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TO OUR READERS

With this issue *Acta Pædiatrica Scandinavica* changes its size, design and typography so that the journal will now answer to international standards. The new typography is space-saving which means that the same number of articles will be printed on a reduced number of pages. This will also improve the legibility apart from being more economic from the viewpoint of production.

Before deciding to make these changes the Board of Trustees discussed the question thoroughly carefully considering the advantages and disadvantages. It is hoped that their decision will also meet with the full approval of the readers. These measures have, however not been sufficient to cover the increasing costs of printing and paper It has also been found necessary to raise the subscription price to Swedish kronor 90. The number of supplements published by *Acta Pædiatrica Scandinavica* has during recent years shown a tendency to increase, in itself a most gratifying fact which, on the other hand, is accompanied by increased expenses. This has been a contributing factor influencing the decision to raise the subscription price.

For several years Ass. Professor Bo Hellström has been responsible for the section of the Editorial Board dealing with book reviews. This is a very difficult and often a most unrewarding part of the editorial work. To find good and willing reviewers is not an easy task but we feel that Dr Hellström has been successful in his work and when he with this issue, retires the Board of Trustees wish to express its sincere gratitude.

Acta Pædiat Scand 57 2–8 1968

METACHROMATIC LEUCODYSTROPHY IN EARLY CHILDHOOD

Treatment with a diet deficient in vitamin A

Johannes C. Melchior and Jørgen Clausen

From the University Clinic of Paediatrics, Rigshospitalet Copenhagen, and The Neurochemical Institute Copenhagen, Denmark

Among the progressive encephalopathies in childhood particularly metachromatic leucodystrophy has been studied, and during the last ten years knowledge has been gained about the pathogenesis of this disorder

The finding of metachromatic deposits in the central nervous system and in the kidney epithelium has been recognized by neuropathologists for many years. But only during the last few years it has been possible to diagnose the metachromatic leucodystrophy during life both by the rather typical clinical picture of four stages and a number of special tests (2, 4 5 13–17).

Already Witte (23) and later Austin (1) described the metachromatic urinary sediment; later the increased excretion of sulfatide was shown (?) and finally the low activity of sulfatase was unveiled (3) Metachromatic staining of biopsies from cortex, rectum, and more frequently used from peripheral nerve (14 22) is of importance for the diagnosis. The nerve conduction has been shown to be delayed in this disease (10 12) The intensive studies have made it clear that the disease is a sulfatide lipidosis (2, 18) leading to storage of sulfatide, because of lack of the degradative lysosomal enzyme (arylsulphatase A) normally cleaving sulphatides

In this communication, the case of a well-established metachromatic leucodystrophy in a child will be presented The given data show an attempt to alter the natural course of the disease by restriction of vitamin A which is necessary for the synthesis of sulfatides.

The studies have been supported by a grant from the Fund for Studies of Mental Retardation, The Danish Ministry of Social Affairs.

CASE REPORT

S. M H. is a girl born in May 1964 as child no. 14.

Family History The maternal great-grandmother had a number of children who died at early ages from disease resembling that of our patient. The parents are both healthy Of the 13 elder siblings to the index patient, four boys have died, but the other nine children are all healthy

Child no. 3 died at the age of 6 months from disease characterized by high temperature and convulsions He was said to be healthy until he became acutely ill and was admitted to the local hospital with temperature of 42°C. H died 30 minutes fter admission. No autopsy was performed.

The three other boys who died, showed an almost identical clinical picture. They developed normally until the age of 18 months when they started to deteriorate gradually by loosing their motor abilities and their ability to talk and had grand mal convulsions. Later they showed signs of spastic tetraplegia and mental retardation. Pains in the extremities occurred periodically often combined with extension spasms. The duration of their fatal disease was from one to two years.

The boys were examined at different times and with different tests, and even if an exact diagnosis was difficult to establish in the beginning, it was later shown to be clear diffuse sclerosis or leucodystrophy This was definitely shown in one case at brain autopsy and suggested in another case from chemical study of a cortical biopsy

The most relevant clinical data and examinations are listed in Table 1

I review in 1960 of 16 cases of diffuse sclerosis the diagnosis f metachromatic leucodystrophy was stated in the brothers (7)

Clinical E aluation. S. M H was delivered normally after an uncomplicated pregnancy Birth weight was 3000 g, and there were no neonatal abnormalities. She developed normally for the first six months, after hich she was able to sit unsupported. Since then her motor development stopped, and she never learned to walk. At the age of two years she was severely motor handicapped with loss of muscle strength in the extremities, and at

Table 1 *Case histories of the three brothers with metachromatic leucodystrophy*

Child	No. among siblings	Pregnancy and delivery	Birth weight (g)	Milestones	Disease diagnosed	Duration of disease	Examinations of importance for the diagnosis of metachromatic leucodystrophy
Boy born Sept. 1950	6	Uncomplicated	3500	Sat alone almost 2 yrs old. Was able to support himself in upright position. Single words at 20 months	2 6/12 yrs old. Grand mal convulsions. Spastic tetraplegia	More than 6 months. Died 2 6/12 yrs old	Brain autopsy metachromatic leucodystrophy. For details see case 8 in Christensen, Melchior & Negri, 1961 (7)
Boy born March 1954	8	Uncomplicated	3500	Walked alone 18 months old. Said single words 12 months old	Loss of motor abilities from 18 months. Grand mal conv. at 3 1/12 yrs. Pains in extremities	2 years. Died 3 6/12 yrs old	Spinal fluid 61 mg % of protein. EEG abnormal curve with low activity. PEG ventricular system slightly dilated. No autopsy
Boy born July 1957	11	Uncomplicated	3000	Walked alone 18 months old	Grand mal conv. 12 months old. From 18 months loss of motor abilities	2 years. Died, 3 3/12 yrs old	Spinal fluid. 83 mg % of protein. EEG normal IQ (Jan 1960) 89 PEG Normal. EMG Normal. Muscle biopsy Myositis. Cortical biopsy no histological examination. Chemical analysis was characteristic for leucodystrophy (C. M. Plum)

this time she developed shortening of the Achilles tendons. She spoke single words when 12 months old, but later she also lost her ability to speak. She was admitted to the local paediatric department 2 $^{1}/_{12}$ years old and it was found that she had flaccid paresis of her legs with bilateral Babinski signs, but without patellar reflexes. A muscle biopsy suggested neurogen disorder. At that time she was transferred to this department for further studies. On the first admission in December-January 1966–1967 she was of normal height and weight. She was able to assist somewhat in acquiring sitting position and was able to sit unsupported for a moment, but it was difficult for her to maintain the position of her head. The muscular tone was decreased and there were no patellar or abdominal reflexes. Bilateral Babinski signs were still present. The tendon reflexes of the upper extremities were weak but symmetrical. Her reactions to painful stimuli were slow and weak. Nystagmus was present on the right eye; eye examinations were otherwise normal.

After this admission she was followed as an out-patient and shortly readmitted in March, 1967 at which time she was unable to sit alone or to assist in acquiring sitting position any longer otherwise the clinical findings were unchanged.

She was unable to speak, but partly understood what was said to her. She had, on both admissions periodical pains in all extremities. Though she never had seizures, she had increasing extension spasms on which Librium® had some effect.

During her admissions a number of examinations were performed, only the most relevant will be mentioned. X-ray of the skull was normal. Cholecystography was normal. The spinal fluid contained 215 mg protein and 48 mg glucose per 100 ml. Fractionated seruмprotein and non polar lipids were normal. EEG was normal. EMG showed a slight loss of motor units and the motor conduction velocity was reduced in several areas to about 12 m/sec. Also the sensory function was impaired. The findings were the same in December 1966 and in March 1967 and suggest peripheral polyneuropathy as seen in metachromatic leucodystrophy. Biopsies from the sural nerve and from the rectum revealed characteristic metachromatic deposits (Erna Christensen).

From December 12, 1966 to May 1967 she was fed a diet low in vitamin A. Other vitamins were given in

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Table 3 *Arylsulphatase activity in urine from the patient S M H with metachromatic leucody   hy*

	A	B	A	B
	μg nitrocatechol released/ml urine/hour		μg nitrocatechol released/mg urine prot/hour	
Normal values				
0–1 year	7 4 ± 1 6	2.8 ± 0.9	47 7 ± 4.9	21 4 ± 5 9
1 5 years	11 1 ± 5 1	7.5 ± 2.6	46.1 ± 13 0	32.3 ± 6.9
5–20 years	11.0 ± 1 6	10 6 ± 2.1	17 8 ± 2.6	16.4 ± 2.5
over 20 years	23.5 ± 4 4	7 7 ± 2.3	52.5 ± 7 8	16.2 ± 3 7
Patient				
12 9 1966	0 0	1.4	0.0	0.7
1 12 1967	2.3	0.8	13.8	4.9
1 25 1967	1 1	5 3	5 0	23.2
2 13 1967	1 8	0.0	6.4	0 0
2 24 1967	1 7	0 7	8.6	3.4
3–10 1967	0.9	0.2	4 0	5 0

BIOCHEMICAL RESULTS

Table 2 shows the pattern of sialic-acid free polar lipids in the urinary sediment of the patient, S. M. H., prior to and during the period of treat ment with a diet deficient in vitamin A. Initially the polar pattern of the urinary sediment was characterized by presence of two predominating glycolipid fractions with *Rf* values of 0.59 and 0.71 These two fractions were both resistent to hydrolysis with KOH (*vide supra*). During the following months (Table 2) of low vitamin A intake the polar lipid pattern was changed. Thus the above mentioned two polar lipid fractions decreased relatively to an increase in polar lipid fractions with *Rf* values below 0.52.

Table 4 *Sialic acid free polar lipids in urinary sediment from family members*

Rf value ...		0.15	0.32	0 43	0 55	0 62	0.72	0 92
Lipid ...	Age (years)	Pa + Sf	Le	Pe	Glycolipid			
Mother (L. H.)	42	—	1.2	27 7	36.2	—	34.8	—
Father (E. H.)	45	7 7	3.9	2.6	20 5	25.7	26.8	12 8
Brother (K. H H)	23	5 6	25 9	7 9	37 2	—	7 9	15 5
Sister (B. L. H.)	22	—	11 1	—	52.8	19.5	16.7	—
Sister (I L. H)	19	2 0	3.9	7 9	39 3	15 7	31 4	—
Sister (K. M H)	14	7 5	3 7	37 0	48 1	3 7	—	—
Sister (K. H.)	12	9.9	2.7	42.3	43.2	—	1 8	—
Brother (T H)	11	11 1	1 4	16.2	53 5	16.2	1 7	—
Brother (T H)	7	6.0	9 7	29 9	47 0	7 5	—	—
Normal values 12 Adult females		45 0 ± 4 6	8.2 ± 1.8	9 6 ± 1 5		37.2 ± 3.6		
Normal values − 6 Adult males		67.6 ± 5.6	7 1 ± 5.6	13 8 ± 1.0		11 4 ± 1 9		
Normal girls < 18 yrs − 12		50 4 ± 3 4	15.0 ± 1.8	13 6 ± 3.4		20 9 ± 5 0		
Normal boys < 18 yrs − 12		63.6 6 8	7 7 ± 1 6	7.5 + 2.5		19 7 ± 4.0		

Table 5 *Arylsulphatase activity in urine from family members*

Italicized figures indicate the values of arylsulphatase A decreased more than two times the standard deviation of the mean

	Age, years	A μg nitrocatechol released/ml urine/hour	B	A μg nitrocatechol released/mg urine prot/hour	B
Normal values					
0–1 year		7 4±1 6	2.8±0.9	47 7±4.9	21 4±5.9
1–5 years		11 1±5 1	7.5±2.6	46.1±13.0	32.3±6.9
5–20 years		11 0±1 6	10 6±2.1	17 8±2.6	16.4±2.5
over 20 years		23.5±4 4	7 7±2.3	52.5±7 8	16.2±3 7
Mother (L H.)	42	*13.9*	5.8	110.3	45.9
Father (E. H.)	45	33.6	12.8	248.8	94 8
Brother (K. H. H.)	23	*14.9*	5.3	60.4	21 6
Sister (B. I H.)	2.	*8.2*	*2.4*	56.8	17 0
Sister (I. L. H.)	19	12.3	5.3	60.4	26.3
Sister (K. M. H.)	14	*5.*	1.2	14.0	3 4
Sister (K. H.)	12	*4.6*	75.7	*12.8*	44 0
Brother (T H.)	11	18.1	0.0	90.3	0.0
Brother (T H.)	7	*2.4*	0 0	*7.6*	0.0

Table 3 demonstrates the arylsulphatase activities of the urine during the same period compared to the normal values. The arylsulphatase-A activity (both the specific activity and the activity per ml urine) was zero or very low and did not change during the period of deficiency in A vitamin.

Table 4 demonstrates the polar lipid pattern of sediment of the relatives. All living ly members had a glycolipid fraction with a *Rf* value of 0.55 and furthermore the fraction *Rf* 0.7. was found in all but one, the father three sisters and two brothers had a glycolipid fraction with a *Rf* value of 0.62. A fast moving fraction 0 92 was noticed in two cases.

Table 5 demonstrates the sulphatase activities in urine from the relatives of the patient. No relatives lacked sulphatase A. However the arylsulphatase A activity expressed as μg nitrocatechol liberated per ml urine per hour was lowered significantly in the urinary specimens from the mother three sisters and two brothers. The specific activity of sulphatase A was lowered significantly in one sister and one brother These alterations were only unveilable when the normal age-dependent values of the different age groups were taken into account (Tables 3 and 5)

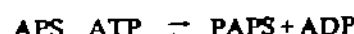
DISCUSSION

Four siblings in a family of 14 children suffered from a disease which in three cases was suspected to be metachromatic leucodystrophy and in one was proven to be this disorder The diagnosis is clinically typical and verified through biopsies from the sural nerve and rectum and supported by electromyography and conduction velocity The biochemical studies in the present communication confirm the clinical diagnosis of metachromatic leucodystrophy by revealing lack of arylsulphatase A in urinary specimens from the patients in question. Furthermore, the screening of polar lipids in urinary sediment confirmed the presence of two predominating polar lipid fractions with *Rf* values in the regions of glycolipids.

The treatment with a diet deficient in vitamin A resulted in a pronounced (five times) reduction of the glycolipid fractions of the patient during four months of treatment. However this treatment did not influence the metabolic lack of sulphatase A. These findings are logical and in keeping with earlier data (21) Vitamin A incorporated in liver tissue seems to act as a coenzyme in the first step in the synthesis of active sulphate:

Vitamin A


ATP + SO ⇌ APS + P P


APS ATP ⇌ PAPS + ADP

The active sulphate (PAPS) is transferred to galacto-ceramide thus forming sulphatide. In metachromatic leucodystrophy the lack of sul-

phatase A causes the accumulation of sulphatide (3 5 18). The vitamin A deficiency in the present case could obviously compensate for the reduced sulphatase activity by lowering the synthesis of sulphatide.

Unfortunately there was no clinical improvement during the four months of treatment. The disease was too advanced and she is now clinically in stage three to four

Nevertheless, it seems possible to influence the abnormal pattern of metabolism and it may be possible to avoid storage of sulfatide in the central nervous system if it is possible to establish an early diagnosis of metachromatic leucodystrophy This means that in families with expectance of affected children one must examine the children as early as possible, repeating these examinations regularly to make a diagnosis. The sulphatide excretion in younger children is rather unspecific (17) and perhaps the determination of sulphatase is more likely to give the answer Also, the biopsy from peripheral nerve, rectum or dental pulp might be a rather late phenomenon, and thus be of little help in the early diagnosis.

In the present case no great change in the condition could be expected as the storage and destruction of the brain had lasted at least one to two years. In the other members of the present family a number of abnormalities were found in the metabolism of sulphatide. Two glycolipid fractions predominated in the urinary sediments of the whole family with the exception of one member Furthermore, two additional fractions were seen in some samples. However the clinical significance of these fractions seems doubtful as sulphatides and other glycoliped fractions are normally occurring substances in urinary sediment and human kidney (17). Great changes occur in these substances during early childhood (Michael & Clausen in preparation). The screening of sulphatase activity of urinary specimens seems of greater clinical significance. The arylsulphatase activities detectable by hydrolysis of nitrocatechol sulphate correspond to the total activity of enzymes catalyzing the hydrolysis of different biological sulphat esters as sulphatides, acid mucopolysaccharides, sulphate esters of phenols, steroids and alcohols. However the present communication as well as previous studies have nevertheless shown that the screening system is of clinical value since the sulphatase A activity is reduced in metachromatic leucodystr phy (3 5 18). The low arylsulphatase A acti found in the mother three sisters and two brothers may reveal these as carriers. Thus the determination of arylsulphatase activity may help to predict carriers of the disease.

SUMMARY

The clinical, biochemical and pathological findings in a family with four cases of metachromatic leucodystrophy are presented. The biochemical studies of one of the cases revealed the absence of urinary sulphatase A and the preponderance of two glycolipid fractions in urinary sediment.

The patient in question was fed a diet low in vitamin A for four months. This diet reduced the relative amount of glycolipids five times in the urinary sediment (relative to the total content of sialic acid free polar lipids). The diet did not change the sulphatase activity However no significant clinical improvement of the patient was noticed. The biochemical changes induced by the deficiency in vitamin A are discussed on the basis of biochemical studies showing vitamin-A to be necessary for the synthesis of active sulphate (PAPS). PAPS is necessary for sulphate incorporation in galactoceramide during biosynthesis of sulphatide. It is suggested that a diet deficient in vitamin A may be used in treatment of metachromatic leucodystrophy

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DERMATOGLYPHS IN CONGENITAL HEART DISEASE

A familial survey of 100 cases

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In two previous papers (8, 9) studies of the finger and palm prints in 150 patients with congenital heart disease have been reported. Statistically significant differences of total print patterns for both finger and palm prints between the various diagnostic groups were found. Finger print patterns were classified as described below and it was found that arches were frequent with pulmonary stenosis, ulnar loops with ventricular septal defect and whorls with aortic stenosis and Fallot's tetralogy. It seemed that these group differences were due to a few of the patients who each had a large number of individual finger prints of the appropiate pattern. It was suggested that these patients could comprise a genetic fraction of the various anomalies and it was thought that a familial study would be of interest to clarify this point. With regards to palm prints, most types of congenital heart anomaly but especially Fallot's tetralogy tended to have the axial (*t*) triradius in a more distal and radial position than do control subjects.

Prior to these papers Hale *et al.* (2) had observed a tendency for a distal *t* position (i.e. broad *atd* angle) in patients with congenital heart disease, but they did not analyse the print pattern according to the type of malformation. Fried & Neel (1) were not able to corroborate these findings but again no diagnostic analysis was attempted. More recently Kontras & Bodenbender (5) have separated their diagnostic groups: their findings were similar to our earlier results; they also suggested the need for a familial study.

In this paper the results of a familial study based upon 100 patients with congenital heart disease are reported. As patients with chromosomal aberrations and those with multiple malformations syndromes do usually show dermatoglyphic abnormalities, only cases with isolated congenital heart disease were included. Furthermore there was no case of parental consanguinity in the series.

MATERIAL AND METHODS

Finger and palm prints were obtained from the proband (Pr), the father (F) and the mother (M) in 100 families. Mid-parent (MP) values were then calculated. All the probands were selected on the basis that they had congenital heart disease, whose diagnosis was soundly established.

The individual finger print patterns were classified as *arch* (no triradius), *ulnar loop* (one radial triradius), *radial loop* (one ulnar triradius) and *whorl* (two triradii). Total finger ridge count (TFRC) was obtained by counting the ridges crossed by a straight line drawn from the triradius to the core of the pattern (arches score 0) and then adding the values for all ten digits.

The palm point was said to be in the t' position if the *atd* angle was 45° or less, t' if it was between 46° and 70° or t'' if it was 71° or more. When more than one triradius was present the *atd* angle was constructed on the most distal one only.

Familial correlations for TFRC and *atd* values were determined.

In an attempt to determine whether the patients with diagnostically characteristic prints differed from the remainder in their familial behaviours, the "genetic fraction" was arbitrarily defined, in accordance with our earlier findings, as

(*a*) Any patient with t' or t'' axial triradius position.
(*b*) Any case of ventricular septal defect with 10 ulnar loops.
(*c*) Any case of pulmonary stenosis with 3 or more arches.
(*d*) Any case of Fallot's tetralogy with 5 or more whorls.
(*e*) Any case of transposition with or more radial loops.

Table 1 *Total finger ridge count (correlation coefficients)*

Pr = Proband MP = midparent value F = father M = mother GF = genetic fraction NF = non-genetic fraction

Cases	Pr/MP	Pr/F	Pr/M	F/M
All	0.708 ± 0.05	0.37 ± 0.08	0.45 ± 0.08	0.09 ± 0.1
GF	0.63 ± 0.13	0.35 ± 0.15	0.63 ± 0.13	0.22 ± 0.16
NF			0.48 ± 0.11	−0.03 ± 0.13

I all 39 cases were included in the "genetic fraction (GF)" the other 61 formed the "non-genetic fraction (NF).

Statistical analyses were performed for the whole series of 100 probands, and separately for the GF and NF groups.

RESULTS

The familial correlation for the TFRC are given in Table 1 The probandus/mid-parent correlation for the whole series was identical with the theoretically predicted value for this, whilst those for Pr/F and Pr/M were close to theirs. There was also, as expected, no correlation between F and M counts.

The genetic fraction" gave Pr/MP and Pr/F correlation coefficients similar to those for the whole series. There was however a higher Pr/M correlation ($r = 0.63$ as compared with 0 48 for "non-genetic fraction") and F/M correlation (= 0.2_ as compared with − 0 03 for the NF)

Table _ shows the correlation coefficients for the *atd* angle. All coefficients were lower than those for TFRC and again a clear increase for the genetic fraction coefficients was found in Pr/M correlations (0 _3 for the whole series, 0 43 for the GF 0.2_ for the NF) and F/M correlations (0 07 for the whole series, 0.12 for the GF and 0 02 for the NF)

DISCUSSION

The mode of inheritance of the TFRC is well known. Parent/child (Pr/F or Pr/M) correlation coefficients are close to 0.5 (3 4 6) whilst the proband/mid-parent coefficient is close to 0.71 ($= 1/\sqrt{2}$) expected value (3). As assortative mating increases hereditary likeness, interparental correlation needs to be low in any series which is bees analysed.

Penrose (7) has found a sib-to-sib correlation for the *atd* angle of 0 37 parent-child correlation of 0._9 and correlation between monozygotic twins of 0.63 These coefficients demonstrate the value of the *atd* angle for heredity tests also, although its genetic determination is not so strong as for the TFRC.

Our previously reported series (8, 9) demonstrated that a proportion of patients with congenital heart disease have dermatoglyphic traits that substantially differ from normal, it was suggested that they might represent a genetically determined fraction of these malformations.

The familial correlations for the whole of the present series (100 patients) are very close to those predicted and observed in a normal population, demonstrating that for these characteristics their genetic determination was normal and that there was no assortative mating between the patients parents.

Following the hypothesis of the identifiability of a genetic fraction of patients, 39 cases were selected as suggested by the earlier series. For these patients the Pr/M and F/M were higher and differed from those in the rest of cases ("non-genetic fraction").

It seems therefore that patients with congenital

Table 2. *atd angle (correlation coefficients)*

Abbreviations as in Table 1

Cases	Pr/MP	Pr/F	Pr/M	F/M
All	0.25 ± 0.09	0.17 ± 0.09	0.23 ± 0.09	0.07 ± 0.09
GF	0.37 ± 0.15	0.17 ± 0.16	0.43 ± 0.14	0.12 ± 0.16
NF			0.22 ± 0.13	0 02 ± 0.13

heart disease who have characteristic dermatoglyphic patterns (the genetic fraction") are in to those patterns more like their mothers than they are like their fathers and differ in this respect from the rest of the patients. The parents of these patients are more alike, in respect of their dermatoglyphs, than are those of the others, as this was not due to consanguineous matings in the present series, this is evidence of some genetic similarity between these unrelated parents.

The main interest of this observation is academic in its demonstration that at least a proportion of congenital heart disease seems to be genetically determined. In addition there could be some practical applications of these findings: dermatoglyphs seem to provide our first opportunity for identifying the genetically determined cases of congenital heart disease and therefore perhaps of providing some guidance to the parents by genetic counseling.

SUMMARY

A familial dermatoglyphic survey has been carried out in relation to 100 patients with congenital heart disease.

Separating the cases with characteristic dermatoglyphic traits from the rest it was found that they were more like their mothers than their fathers and than the remaining cases. The parents were also more alike in the same respect than the other parents.

These findings support the concept of a genetically determined fraction amongst all the patients with congenital heart disease.

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VENOUS OCCLUSION PLETHYSMOGRAPHY IN CHILDREN

I Method

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A standard method of measuring limb blood flow is that of venous occlusion plethysmography (2) There are many descriptions of the technique and many types of plethysmograph are described (1 4 6, 7 9). Measurement of limb blood flow in children is difficult since this technique requires complete subject co-operation, and the cumbersome apparatus needs careful adjustment and attention during use.

All these techniques use a plethysmograph chamber fitted to the limb and filled with circulating thermostatically controlled water Inflation of a proximal occlusion cuff prevents venous return without impeding arterial inflow The resultant expansion of the limb in the chamber fluid displacement and this is recorded mechanically optically (8), or electrically (3)

Electrical methods which sense alterations in fluid level without any mechanical components are to be preferred. Those described, however will only detect a volume change of a few cubic centimetres. This means that the base line has to be set so that fluid volume change takes place over the linear region.

The apparatus described here is designed for use with children and is suitable for use in the 5–10 year age range. The apparatus has been modified for easy acceptance and to minimize the effect of restlessness. An automatic cycling unit has enabled the apparatus to function independently allowing the operator to be with the subject.

DESCRIPTION OF APPARATUS

The plethysmograph (Fig. 1) is of classical design and is made of a Perspex cylinder 11.5 cm in diameter 12.7 cm long. Perspex annuli fitted with set-screws are sealed to each end. The latex rubber sleeve surrounding the limb is attached to stiff rubber (3.2 mm thick) flanges which fit over the set-screws. Diametrically split annuli, the same size as the rubber flanges, complete the support for the limb and rubber sleeve, and bear onto the Neoprene "O" rings.

In order to simplify the plethysmograph chamber a thermostat and pump were omitted, and these were replaced by approximately 275 cm of 0 64 cm diameter copper tubing wound throughout the inner length of the chamber and brought out to the exterior Thermostatically controlled water is circulated through this tubing from a remote water bath and a by-pass is used to fill the chamber It was found that adequate agitation of this fluid was maintained by the periodic inflation of the proximal occlusion cuff This cuff situated immediately adjacent to the chamber displaces tissue into the chamber when it is inflated. This causes a large fluid volume change,

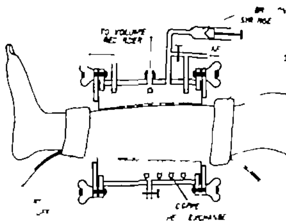


Fig 1 Sectional diagram of the Plethysmograph.

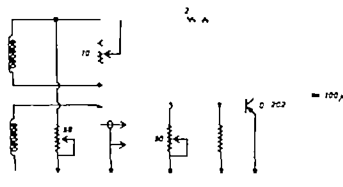


Fig 2 Bridge/Amplifier for sensing changes in conductivity of the level recorder. The circuit is balanced initially with R 1 and R.2 is adjusted for sensitivity. Small changes in base line can be compensated by R.3.

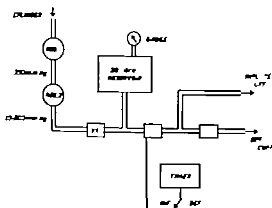


Fig 3. Layout diagram of the Cuff Inflation Apparatus. Reg. 1 is B.O.C. M 30-OG 2 stage regulator. Reg. 2. is single stage latex diaphragm regulator. V.1, V.2 and V.3. are 24 volt oil dilution valves.

recorded as the cuff artefact (7). This method of temperature control and mixing maintains a constant temperature, with no evidence of thermal layering which would require the use of a stirrer.

The juxtaposition of the proximal occlusion cuff gives rise to a large cuff artefact but allows a very accurate estimation of blood flow as only a negligible quantity of blood accumulates outside the plethysmographic chamber. With previous electrical sensing devices it became difficult to compensate for the artefact height in order to allow the subsequent rise in volume to remain on the linear region. We have modified the level recorder to be linear over 5–7 ml and this we have found to be adequate. For very large changes seen in reactive hyperaemia a capacitative level recorder was used with a 15 ml linear range (5).

Here a simple level recorder consisting of two concentric cylindrical electrodes of stainless steel was used. The outer electrode is steel gauze in a Perspex sleeve and the inner electrode is a 6.4 mm steel rod. Changes in conductance resulting from varying heights of the water are detected by a bridge circuit (Fig. 2). This system is electrically safe.

The collecting cuff is inflated by means of an automatic cycling unit and timer (Figs. 3 and 4). Both inflate and deflat periods are variable from 5 to 20 seconds with a manual over-ride. To give maximal speed of cuff inflation the choice of

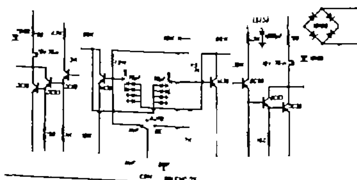


Fig 4 Automatic Timer Unit. The output of this unit operates the solenoids of V.1 V.2 and V.3

tube bore was necessarily a compromise between restriction in air flow and dead space in the tubes. 9.5 mm bore polythene tubing was used. Pressure stability is obtained by means of a 20 l reservoir which was automatically topped up during the deflate" period only

The design of the automatic device eliminates overlap in the solenoid operation which causes instability in pressure supply This unit provided for a few milliseconds delay in the operation of the solenoids to eliminate this effect.

Once the apparatus has been set up long sequences of flow measurements are possible without the need for any attention from the operator who may then devote his time to the patient.

SUMMARY

This paper describes a simple acceptable plethysmograph for use with children which is operated automatically and is not effected adversely by subject non-cooperation. The operating mechanism, thermostatic control and recording apparatus are remote from the plethysmograph chambers, two of which are used for bilateral flow measurements.

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VENOUS OCCLUSION PLETHYSMOGRAPHY IN CHILDREN

II Resting calf blood flow in healthy children

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The measurement of blood flow by venous occlusion plethysmography is not normally carried out in paediatric clinical practice. It is, however, an accurate method of estimating blood flow which causes little or no discomfort (4).

The effect of age on calf blood flow of young adults and the elderly has been studied by Allwood (2), and measurements have been made on the newborn by Celander (5).

With the apparatus previously described (8) we have measured resting calf blood flow in a group of children between the ages of 5 and 11 years.

PROCEDURE

A group of 36 children between ages 5 years 11 years were studied, providing total of 297 measurements. These children are ambulatory in-patients receiving no medication and suffering from no illness associated with circulatory changes.

They were brought to the laboratory dressed in their normal in-door attire, hours after meal. The laboratory temperature was maintained at 20°C ± 0.5°C.

The children were rested for period of 15 minutes whilst apparatus was explained and fitted.

The collecting cuff was placed immediately adjacent to the plethysmograph chamber below the knee. The plethysmograph is positioned on level with the angle of the manubrium sterni. The temperature of the plethysmograph is 34°C ± 0.5°C.

The arterial cuff was inflated and the collecting cuff pressure giving maximum recorded inflow was established. Values ere taken at this pressure after the arterial cuff had been inflated for 5 minutes.

Calf volume as determined by water displacement within the plethysmograph.

RESULTS

See Table 1 The overall mean of 3 4 ml/100 ml/min was obtained from 297 observations on 36 children. The mean result for each age group lies within 1 s.D. from the overall mean, and for the number of observations made, shows no trend with age. Repeat values were n agreement within 10%.

DISCUSSION

Table 2 shows values for calf blood flow obtained by various workers. The results obtained here appear to be in general agreement in spite of the different experimental conditions used.

Celander (5) has considered whether the blood flow in neonates may be raised because the total flow is influenced by different calf composition. Our results indicate that within the 5–11 year age group, any difference that may be encountered in blood flow with age as a result of different calf composition, falls within the limits of experimental variation. Thus a general comparison can be made between calf blood flows at different ages.

Whilst there are large individual variations in calf blood flow simultaneous measurements made with independent apparatus gave results within 5% of each other and repeat observations greed within 10%. Thus more emphasis may be given to changes in an individual than to the difference between individuals or groups of individuals.

SUMMARY

Resting calf blood flow was measured in 36 children between the ages of 5–10 years. The overall mean was 3 4 ml/100 ml/min. The mean for each age group lies within 1 s.D. from the mean.

Table 1 *Calf blood flow in healthy children in one year age groups*

Overall mean = 3.38 ± 1.32 (S.D.), ± 0.22 (S.E.M.)

Age group (years) —	5–6		6–7		7–8		8–9		9–10		10–11	
	Flow	Obs.	Flow	Obs.	Flow	Obs.	Flow	Obs.	Flow	Obs.	Flow	Obs.
Flows ml/min/100 ml tissue	3.9	5	1.9	4	2.3	5	4.2	8	3.7	3	1.4	10
	4.2	4	4.0	7	2.6	5	3 6	3	4 1	3	2.7	8*
	3.6	11	4.9	5	4.3	8	1.9	6	6.3	5	2.1	10*
	2.2	14	4.3	12	3 4	7	3 0	5	2.1	8*	1.5	8*
			5.2	6	2.0	7	3.0	6			5.6	10*
			5.2	7	1 3	14*	2.4	12*				
			6.0	4			3.9	5				
			3.4	18*			2.6	37*				
			3.0	7								
N of cases	4		9		6		8		4		5	
Total Obs.	34		70		46		82		19		46	
Mean	3.48		4.21		2.63		3.09		4 03		2.64	

Denotes bilateral observation.

Table 2. *Calf blood flow values in different age groups*

Source	Age	Water temp.	Cuff	Mean flow (ml/100 ml/min
Celander (5)	1 day	32 C	—	7 9 ± 1 7 } Foot and calf
	7 days	32 C	—	7 3 ± 1 4 } Foot and calf
Alwood (2)	18–.4 years	34 C }	Above knee	2.1 ± 1 2 (S.E.)
	70–82 years	34 C }	Above knee	1 7 ± 1.2 ( )
Winsor & Payne (9)	40 years	—	Above knee	4 0
Dahn (6)	Adults	—	Above knee	3.01 ± 1.35 S.D.
nder (3)	Adults	43 C	Above knee	3 9 ± 1 1
Hillestad (7)	Adults	32 C	Above knee	3 6 ± 1.3
Abramson & Ferst (1)	Adults	32°C	—	1 38 ± 0.5 S.D
Voyce & Preece	5–11 years	34 C	Below knee	3.38 ± 1 3 S.D.

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EFFECT OF PHYSICAL TRAINING ON ADOLESCENTS WITH SEVERE MOTOR HANDICAPS

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Individuals with various forms of physical and mental handicap run a great risk of being inactivated motorically as a result of their motor-handicapping disease, which contributes to a low working capacity (4, 10, 11). This can be partly caused by a low functional capacity of the respiratory and circulatory organs. In healthy subjects, the physical working capacity can be raised by physical training (6, 8), and this has also been found to apply to motor-handicapped adolescents with cerebral palsy whose maximal oxygen uptake increased by 10–15% after 6 weeks training (10).

The effect of physical training on severely motor-handicapped adolescents is, however, unknown. In order to investigate the relevant problems, we made a study before and after a period of physical training in a group of schoolchildren who were so motorically handicapped that a wheel-chair was required, and who could therefore perform work with their arms only.

CASE MATERIAL

The case material consisted of 17 pupils at the Norrbacka Institute Schools (Table 1). All of them needed a wheel-chair for their daily activity. They were divided into two groups on the basis of the diagnosis.

The first group—the cerebral palsy (CP) group—(nos. 1–7) consisted of 7 pupils, 2 boys and 5 girls. The median age was 19 years. Three were in special classes (IQ 65–85) and the others in ordinary classes. Three of them had spastic tetraplegia, 3 variations in muscle tone (athetosis) and one had spastic diplegia.

The other group—the paraplegia group—(nos. 8–17) consisted of 10 pupils with paraplegia of other aetiology than cerebral palsy. There were 4 boys and 6 girls; the median age was 17 years. T of them attended special classes and the others ordinary classes. In 7 cases paraplegia was caused by myelomeningocele, in the other 3 by trauma, tumour and encephalitis, respectively.

All the pupils in the CP group fell within the limits of the ±2 standard deviations in Karlberg & Ingboom growth diagram for healthy Swedish children (7). On the other hand, 8 of the 10 pupils in the paraplegia group were overweight in relation to their height, since they fell outside these limits.

The CP group had a relatively greater motor handicap than the paraplegia group in view of the fact that 6 of the 7 had varying degrees of motor disturbance in their arms. This did not apply to any of the pupils in the paraplegia group. The difference in functional capacity is illustrated by the differing ADL (activity of daily living) ability in the two groups. Thus, help with dressing and undressing was needed by 4 of 7 CP pupils, but by only 4 of 10 in the paraplegia group. T of the CP pupils could not drive their wheel-chair without assistance, whereas this could be done by all those in the paraplegia group. None of the pupils in either group required help at meals. Those in the paraplegia group were able to drive their wheel-chairs faster than those in the CP group, because of the impaired function in the arm muscles of the latter.

METHODS

Before the exercise test, all the pupils underwent a conventional physical examination, including recording of the ECG at rest with precordial leads. Weight and height were recorded. In several cases, the height had to be measured sitting in a wheel-chair in view of the inability to stand upright. The height was then taken as the sum of the measurements from the crown of the head to the proximal end of the thigh, thence to the kneecap and finally to the lower surface of the heel.

All the pupils had, before the exercise test, trained working with their arms on the bicycle ergometer so as to become familiar with the procedure.

Before the training period, all had carried out submaximal work with their arms for 6 minutes, the load being chosen so that the heart rate during work was above 130 beats/min. The same submaximal work was performed after the end of the training period. Since

This study was carried out with a grant from the Folke Bernadotte Foundation for Children with Cerebral Palsy

Table 1 *Case material*

I cases 1 2, 8 and 17 the height could be measured in the upright position in the other cases it was measured sitting in wheel-chair

Case	Sex	Age (yrs)	Height (cm)	Weight (kg)	Class*	Diagnosis
1	F	19	158	59.5	S	Tetraplegia
2	F	19	154	45 4	O	Tetraplegia
3	F	18	162	52.5	S	Athetosis
4	M	24	171	52.3	O	Athetosis
5	F	18	152	49 8	O	Diplegia
6	M	18	164	46.9	O	Tetraplegia
7	F	19	145	37 8	S	Athetosis
8	M	17	152	67.8	S	Myelomeningocele
9	F	17	145	55.4	O	Myelomeningocele
10	M	14	152	66 8	O	Traumatic spinal cord damage
11	M	20	174	88.5	O	Tumour
12	F	16	148	57 0	O	Myelomeningocele
13	F	15	148	46.5	O	Myelomeningocele
14	F	20	128	36.0	O	Myelomeningocele
15	F	18	139	55 6	S	Myelomeningocele
16	F	18	160	54 4	O	Encephalitis
17	M	17	149	55 0	O	Myelomeningocele

S = Special class; O = ordinary class.

some pupils had difficulty in maintaining the desired pedal rate of 50 rpm, this was checked with tachometer on the bicycle ergometer The work load was then calculated from the pedal rate and dosed load.

After the submaximal ork had been followed by at least 5 minutes rest and "warming-up period of 3 minutes, 8 of the pupils in the paraplegia group per ed maximal ork, which xhausted them after 3–6 minutes. After the end of the training period, this maximal load was increased somewhat, in iew of the raised orking capacity

The expired air was collected during the last 2 minutes of the exercise test.



Fig 1 Bicycle ergometer for arm work. Inset, detail of scale.

The heart rate was recorded on the ECG during each minute of the test. The rate is given as the mean for the 5th and 6th minutes.

From 1 to 3 blood samples for determination of the lactic acid content were taken within 1 5 minutes of ending the test. The highest of these values is given.

The heart volume was determined roentgenologically on day when the exercise test was not made.

All the *exercise tests ere made on a mechanically* braked (Monark) bicycle ergometer (5) Its construction had been altered so that the pupils could carry out arm work with the pedals while sitting in a wheel-chair (Fig 1). A technical modification permitted lower loads, i.e dow to 0.25 kg.

The oxygen uptake was measured by the Douglas bag *method. The volume of the expired air was measured in* balanced spirometer The gas was analyzed by Haldane method

The ECG was recorded with single-channel electrocardiograph (Siemens). The heart rate was determined by calculating at least 15 cardiac cycles

The blood content of lactic acid was determined by the method of Baker & Summerson (3), as modified by Strom (13).

The heart volume was determined roentgenologically in the recumbent position (9).

Con entional statistical methods were pplied, and the significance level expressed by p 0 05

Training was carried out for 30 minutes twice ek for 6 weeks, during April and M y 1966, concurrently with the usual school gymnastics 2 3 times ek. The training was intended to involve as large as possible muscle groups continuously for 2 5 minutes. This was achieved by rapid heel-chair driving, throwing medicine

Table 2. *Results. CP group submaximal load*

B = Before training; A = after training; $\bar{x}$ = mean of individual observations; p = gives the degree of signif. between A and B

	Load (kpm/min)		Oxygen uptake (l/min)		Heart rate (beats/min)		Blood lactate (mg/100 ml)		Heart volume (ml)	
Case	B	A	B	A	B	A	B	A	B	A
1	75	75	0.81	0.83	160	159	40	35	530	355
2	105	105	0.81	0.87	189	190	54	73	380	390
3	150	150	0.84	0.82	178	153	51	33	400	390
4	100	100	1.08	0.97	165	148	46	21	420	475
5	75	75		—	103	108	27	16	290	350
6	75	75	0.70	0.70	159	171	40	17	360	445
7	75	75	0.41	0.49	170	126	[b]	—	470	435
$\bar{x}$			0.775	0.783	153.4	150.7	43.0	32.5	407.1	404.3
p			> 0.05		> 0.05		> 0.05		> 0.05	

[a] Measurement not made, due to pupil's aversion to mouthpiece.
[b] No measurement, due to technical mishap.

balls, using dumb-bells, levering movements in wheel-chair and on parallel bars. In the intervals (30 sec to 2 min) between the various exercise shifts, the pupils drove their wheel-chairs continuously which corresponded to the jogging which occurs during ordinary training.

During the last few minutes of various training procedures, the heart rate was determined on tw occasions in 5 pupils in the CP group and in 7 in the paraplegia group. The average pulse rate on these tw occasions was 115 beats min in the former group and 140 beats/min in the latter.

The average attendance at the training sessions was 11 out of 12 possible times, with no difference between the groups in this respect. The pupils interest in the training was noted, and in all but one case in each group was extremely good.

RESULTS

The individual values before starting training, as well as after ending it, are given in Tables 2, 3 and 4 and Figs. 2 and 3.

Table 3. *Results: Paraplegia group submaximal load*

For symbols, see Table 2

	Load (kpm/min)		Oxygen uptake (l/min)		Heart rate (beats/min)		Blood lactate (mg/100 ml)		Heart volume (ml)	
Case	B	A	B	A	B	A	B	A	B	A
8	300	300	1.12	1.02	162	136	53	23	650	810
9	135	135	0.53	0.48	160	135	18	22	390	430
10	150	150	0.83	0.75	145	137	25	16	560	330
11	225	225	0.96	0.73	182	175	42	28	610	660
12	150	150	0.68	0.61	168	150	34	26	530	480
13	135	135	0.49	0.51	179	137	59	33	400	460
14	120	120	0.47	0.42	—	—	84	44	320	230
15	75	75	0.48	0.48	135	131	27	18	380	345
16	150	150	0.63	0.56	132	112	36	16	390	420
17	150	150	0.83	0.77	150	140	—	—	—	—
$\bar{x}$			0.702	0.633	157.0	139.2	42.0	25.1	470.0	462.8
p			< 0.01		< 0.01		0.01		> 0.05	

[a] Heart rate not measurable, due to corset.
[b] No determination, due to technical mishap.

Table 4. *Results Paraplegia group maximal load*

For symbols, see Table 2

Case	Load/time (kpm/min min/sec) B	Load/time (kpm/min min/sec) A	Oxygen uptake (l/min) B	Oxygen uptake (l/min) A	Heart rate (beats/min) B	Heart rate (beats/min) A	Blood lactate (mg/100 ml) B	Blood lactate (mg/100 ml) A
8	$475/4^{10}$	$600/3^{38}$	1.54	1.61	182	176	94	68
9	$225/3^{43}$	$210/3^{10}$	0.72	0.60	190	178	12	64
10	$375/3^{40}$	$396/3^{38}$	1.29	1.25	197	186	83	64
11	$450/2^{44}$	$563/4^{00}$	1.25	1.26	189	196	78	70
12	$200/3^{54}$	$414/3^{00}$	0.97	0.99	194	178	70	50
15	$175/4^{00}$	$216/4^{00}$	0.65	0.78	182	169	53	51
16	$330/3^{00}$	$330/6^{00}$	1.06	0.98	173	162	69	65
17	$390/3^{1}$	$440/5^{15}$	1.25	1.35	189	189	83	85
$\bar{x}$	$327.5/3^{31}$	$396.4/4^{18}$	1.091	1.103	187.0	179.3	74.0	64.6
p	< 0.01		> 0.05		< 0.05		< 0.05	

CP Group

No change in the oxygen uptake during exercise with a submaximal load was noted after training. The average heart rate was 3 beats/min less after training. The average blood lactate concentration was 43 mg/100 ml before training and 33 mg/100 ml after it ($p > 0.05$). The heart volume was an average 407 ml before training and 404 ml after it.

Paraplegia Group

Significantly lower values for the oxygen uptake were noted after training than before it, the average being 0.63 and 0.70 l/min, respectively ($p < 0.01$). The heart rate had fallen by 18 beats/min ($p < 0.01$), and the blood lactate had decreased significantly from 42 to 25 mg/100 ml ($p < 0.01$). No significant difference was demonstrable in the heart volume before and after training (average 470 and 463 ml, respectively).

The 8 pupils who were able to carry out maximal arm work (nos. 8–12, 15–17) did this before training with an average load of 340 kpm during 3 min and 31 sec. Compared with 396 kpm during 4 min and 18 sec after training, this implies an increase of 40% in the total work. The oxygen uptake during maximal work was unchanged after training, whereas the heart rate had fallen by an average 8 beats/min ($p < 0.05$). The blood lactate was also significantly lower after training ($p < 0.05$)

DISCUSSION

From the physiological point of view arm work differs in several respects from leg work. Thus, with the same submaximal load, the oxygen uptake, heart rate and blood concentration of lactic

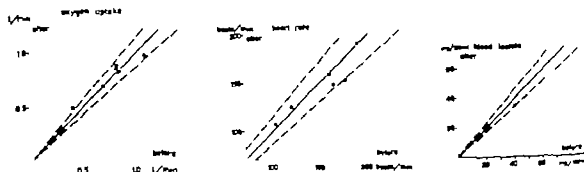


Fig. 2. Results: CP group. Submaximal work. (The broken lines denote the ±10% deviation from the line of identity.)

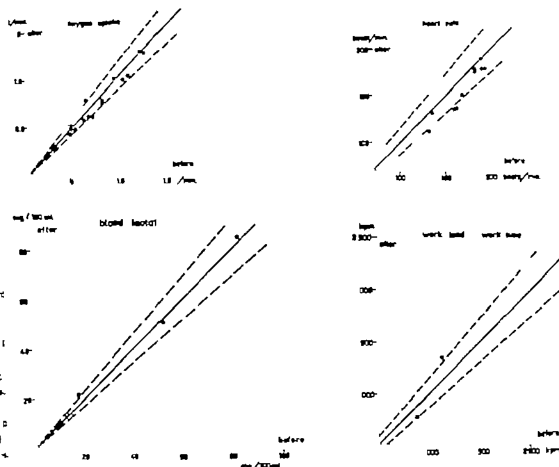


Fig. 3. Results: Paraplegia group. (Unfilled symbols represent values with submaximal work loads, filled symbols values with maximal work loads. The broken lines denote the ±10% deviation from the line of identity.)

acid are higher during work with the arms (1 1.). In healthy experimental subjects, the maximal oxygen uptake during arm work is about one-third of that during leg work (2). Consequently the results of the present study are not fully comparable with those of other studies in which work was performed with the legs.

Despite the intensity of training being relatively low and the fact that it was carried out for fairly short time—6 weeks—an effect was noticeable in most pupils, particularly in the paraplegi group. In the CP group the visible effect of training was an average decrease in blood lactate (by 23%); it was not, however significant for the whole group. Case 2 had a 19 mg/100 ml higher blood lactate concentration fter training than before. When the test was made after training, her pulse rate at work was 190 beats/min, which implies that she actually performed almost maximal work, in contrast to the other pupils in the CP group. If only the 5 pupils who carried out submaximal work are taken into account, the difference is significant ($p < 0.05$).

The effect of training was more marked in the paraplegia group. This difference can be explained by the difference between the two groups with respect to the total training work performed. A relatively lesser degree of motor handicap in the paraplegia group allowed a greater intensity of training than in the pupils with cerebral palsy. The average pulse rate during training was, in fact, 25 beats less per minute than in the paraplegia group (115 and 140 beats/min, respectively).

The mechanical work efficiency had improved in the paraplegia group, which is evident from

the decreased oxygen uptake during submaximal work. The lower heart rate after training is explained partly by the reduced oxygen uptake, and partly by an improvement in cardiovascular function.

As in the CP group the blood lactate concentration fell after submaximal work, indicating a more effective oxygen supply to the exercising muscles.

In maximal work in the paraplegia *group it was found that the total work performed could* be increased by 40%. This was due in part to a prolonged working time, and in part to the ability to increase the work load. The maximal oxygen uptake was, however unchanged after training, which indicates an increased mechanical work efficiency during maximal work, moreover the heart rate and blood lactate concentration also showed lower values after training. It therefore seems probable that the pupils were unable to utilize their total oxygen uptake ability and anaerobic work power during the maximal work after training. The reason is possibly to be sought in inadequacy of their trunk muscles, which failed to give their body the stability which is a prerequisite for carrying out maximal work with the

[illegible] this moderate training produced good [illegible]ts in these severely motorially handicapped adolescents, it can be assumed that their ordinary gymnastics had been inadequate for intensive training of their cardiovascular system, and for producing the best possible effect in view of their motor handicap We therefore consider it important for the conventional gymnastics for motorially handicapped pupils to be complemented by training procedures of the simple nature described in the aforegoing. This is in order to produce the desired increase in their working capacity which is presumably essential for their achievements both in their school work and during their free time. In our opinion, these adolescents—who show signs of definite initial inactivation—possess good reserves that are available for mobilization. Furthermore, training of the kind described is an important factor in habilitation of these motor-handicapped young people, in addition to the conventional methods of treatment for children and adolescents with motor handicaps.

SUMMARY

The case material consisted of 17 pupils at the Norrbacka Institute Schools. Cerebral palsy (CP) was present in 7 cases (mediam age 19 years), and paraplegia of other origin—chiefly myelomeningocele—in 10 (median age 17 years). All had severe motor but not mental handicaps, and had to use a wheel-chair for their daily activities

Physical training was carried out for 30 minutes twice a week for 6 weeks, concurrently with the ordinary gymnastics. It consisted of fast wheel-chair driving, exercising with medicine balls and dumb-bells, and levering movements in a wheel-chair and on parallel bars.

Exercise tests were made on an ergometer bicycle for arm work, the loads were submaximal for all pupils, and also maximal for 8 in the paraplegia group Identical submaximal tests were carried out before and after the training period, together with determination of the oxygen uptake, heart rate, blood lactate concentration and roentgenological heart volume.

In the CP group, the blood lactate concentration was lower after training than before it. In the paraplegia group, significantly lower values were recorded after training for oxygen uptake, heart rate and blood lactate. No change in heart volume was detected in either group.

In the 8 pupils in the paraplegia group who could perform maximal work, a 40% increase was noted after training.

The better training results in the paraplegia group can be explained by the more severe handicap in the CP group, in which the arms were also involved in most cases. The heart rate during training was an average 140 beats/min in the paraplegia group, but only 115 beats/min in the CP group.

The results show that these severely handicapped adolescents were able to improve their working capacity despite training being of fairly mild intensity and carried out during a relatively short period. This implies that the ordinary gymnastic lessons were inadequate for improving the pupils working capacity and should therefore be complemented by a training programme of the type described. A better working capacity is highly desirable for their performance at school, as well as during their activity of daily living.

It is concluded that this type of training should be included in the conventional habilitation programme for children and adolescents with motor handicaps of this type.

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HEREDITARY FRUCTOSE INTOLERANCE IN FOUR SWEDISH FAMILIES

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Hereditary fructose intolerance (HFI) is characterized by nausea, vomiting, pallor, tremor, drowsiness and sometimes coma on taking fructose. The condition was first observed in 1956 by Chambers & Pratt (3).

In 1957 Froesch *et al.* (10) presented four cases in one family, described the symptoms and signs of the condition and showed that administration of fructose caused hypoglycaemia in these subjects. They ascribed the condition to the absence of an enzyme in one of the steps of fructose …olism via the fructose 1-phosphate triose …

In 1961 Hers & Joassin (16) succeeded in demonstrating markedly diminished 1-phosphofructaldolase activity in liver biopsies from patients with HFI, as also Nikkilä *et al.* (29) and Froesch *et al* (13) have since done.

To date the condition has been described in forty-four subjects from twenty-three families; *half are children* (3, 4, 7, 8, 10, 11, 13, 14, 18–22, 25, 26, 31, 34, 36, 42). Grave illness has been described among infants that have received fructose over long periods; mental retardation has been reported (10), and one death has occurred (36). The disease is inherited by an autosomal recessive gene (10, 13); in some families a dominant form appears to occur (4, 26, 42).

Dormandy & Porter (6) have described an aberrant form in two sisters with combined fructose and galactose intolerance.

This paper deals with a description of 7 cases of HFI from four Swedish families. Some studies on utilization of fructose in relation to fat metabolism are presented.

SHORT OUTLINE OF METABOLIC DISTURBANCE IN HEREDITARY FRUCTOSE INTOLERANCE

Enzyme defect. Most of the ingested fructose is metabolized in the liver (24). Fructose is phosphorylated in the liver cells to fructose-1-phosphate by the action of fructokinase. The scission of fructose-1-phosphate takes place with the aid of 1-phosphofructaldolase, which is present in the liver and also in the jejunal mucosa (28) and the kidneys (41). Enzyme studies on liver biopsy specimens from patients with HFI have disclosed abnormally low 1-phosphofructaldolase activity (0–12% of normal) (13, 16, 20–22, 25, 28, 29, 34) and varying but somewhat less diminished di-phosphofructaldolase activity. The 1-phosphofructaldolase deficiency leads to a damming up of fructose-1-phosphate in the cells of the liver (28, 32), intestinal mucosa, and probably also the kidneys (28).

Hypoglycaemia. Froesch *et al* (11, 13) assume that the accumulation of fructose-1-phosphate in the liver cells secondarily inhibits one or more enzyme systems in glycogenolysis or gluconeogenesis, and that this would explain the hypoglycaemia of HFI that occurs on taking fructose. A transient 1-phospho-fructaldolase deficiency may be the cause of the hypoglycaemia that fructose administration provokes in healthy neonates during the first days of life (37).

Hypophosphataemia. In HFI fructose administration brings about a marked, prolonged fall in the inorganic serum phosphate (11), probably owing to the binding of phosphate as fructose-1-phosphate in the liver.

Kidneys. Fructosuria occurs only after taking fructose, and is moderate. It is due to the fructosaemia, and not to kidney damage (13, 34). General aminoaciduria and proteinuria, an expression of tubular damage due to deficiency of 1-phospho-fructaldolase, has been described following prolonged fructose intake among infants with HFI (7, 19–[illegible], [illegible], [illegible], 34, 36). These changes are apparently reversible, and disappear when fructose is eliminated from the diet. Histological investigation in our

case has shown granulovacuolar changes in the tubules (36). Transient aminoaciduria and proteinuria has been described in older children and adults in connection with fructose tolerance tests (11, 18 26)

Liver Enlargement of the liver has been described in 19 of 44 cases (7 11, 14, 19 20, 22, 25 31 34 36, 42), all of them children. Among the infants the enlargement has been marked and some have also shown signs of cirrhosis and splenomegaly. Jaundice was present in five patients, four of them were under six months of age. Increase in transaminase (25 26) and hyperbilirubinaemia (11, 18) were noted during fructose loading, especially by mouth. Liver biopsy was performed in 11 cases and showed fatty infiltration and/or cirrhosis (19–22, 29 34, 36). The hepatomegaly apparently recedes in most cases after elimination of fructose from the diet.

Digestiv tract The violent intestinal symptoms, ith nausea and severe vomiting following ingestion of fructose are not to be classed among the hypoglycaemic manifestations of HFI, but are probably due to the same enzyme defect that has been found in the liver and renal tubules (13). A low content of 1-phosphofructaldolase has then been shown in the jejunal mucosa (7 28). On intravenous administration of fructose nausea and vomiting do not occur which suggests that these symptoms may be due to the local effect of accumulated fructose-1-phosphate (13). Intravenous injection of glucose immediately checks the hypoglycaemia but not the intestinal symptoms.

MATERIAL AND METHODS

The first case came to our knowledge owing to mother' orry that her first child might have "diabetes" like its father (case 1), paternal aunt (case 2), and paternal grandmother (case 3, Fig. 1). At the age of one year this child had tw attacks suspiciously like hypoglycaemia but two oral fructose tolerance tests gave normal results, and the child continued to develop normally. The medical history of the family called for investigation, however. Through case 3 we learned of cases 4 and 5, residents in the same district. A dentist on seeing case 3, recalled man (case 6) that he had previously noted owing to the absence of caries and curious diet. Case 7 as sent to us by colleague W have knowledge of two more patients with characteristic medical history from fifth family not yet investigated.

One healthy student, two healthy pupil nurses and the father of cases 1 and 2 have acted as controls.

Fructose tolerance tests were performed by intravenous administration of 20% fructose over period not exceeding 5 minutes. Fructose and glucose were determined in samples of capillary blood, fructose by Heyrowski method (17) and glucose with the aid of glucose oxidase using Kabi' test kit. Samples of venous blood were collected via plastic catheter inserted in cubital vein about 20 minutes before the fructose test dose. Serum inorganic phosphorus was determined by modification for Technicon Auto Analyzer (N-4 A, 1963) of the Fiske & Subbarow method (9). FFA in plasma were determined by Dole method (5) Plasma gly s measured by Wieland' enzymatic method (n cholesterol was determined by Sackett meth ructose in urine was determined in a two-hour p ected during the fructose tolerance test, usir wki method (17) The urinary adrenaline by the method of Bertler *et al.* (1), modified b tal (15) as for the column procedure

CASE HISTORIES

Case 1 (L. A.), male, born at term in 193., tw h children (Fig. 1). This patient had been breastf ten months. Attempts at weaning to sweetened formul produced nausea, vomiting, pallor and exhaustion refused the formula but accepted ordinary milk T mother realized that the boy like herself could not sugar and gave him the diet she herse! tolerated T infant quickly developed distaste for all sweetened o. If as child he drank bottle of sweetened minera ould ithin half-an-hour omit and remain pale, sh and weak for several hours. During stay at children holiday camp he had an unpleasant eel until the staff finally realized he as telling the truth hen he said h could not take sugar

On examination the patient as found to be an intelligent lean man of normal height. He had no li er enlargement and no dental caries There as no proteinuria and no glucosuria

Determination of serum glucose, fructose, phosphorus (Fig. 2), FFA (Fig. 6), and cholesterol ere done and in this case also the urinary adrenaline.

Case 2 (B L.), female born at term in 1934 sister to case 1. She was breastfed for six months. Dietary supplements of juice and purée produced vomiting, as did sweetened feeds on subsequent weaning. The mother who clearly recognized the symptoms, gave her daughter the same diet that she and the boy could tolerate and the girl thrived As long as she can remember she has disliked all sweet food, and has avoided it scrupulously Like her mother she can tak small quantities of sugar and fruit, but hole orange ill provoke severe nausea, vomiting, abdominal pain, tremor and exhaustion

On examination the patient as found to be of normal build and height and of normal fatness There as no liver enlargement or dental caries (N B her father had only false teeth) There was no proteinuria or glucosuria.

Determination of serum glucose, fructose, phosphorus (Fig. 2), FFA (Fig. 6), glycerol (Fig 7) and cholesterol ere carried out

Case 3 (A A.). Female, born at term 1904 Her parents, sibs, their children, and her own husband ere healthy T children (cases 1 and 2) She had been breastfed for eight months. She had reacted ith vomiting to at tempts at weaning, but know no details. For as long as she can remember she has had distaste for sweets, cakes, fruit, honey and most vegetables all of hich she has avoided She remembers as child eating unsweetened oatmeal porridge hen her brothers and sisters had fruit puddings. She can now tolerate small amounts of sugar

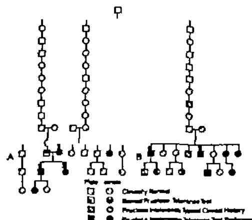


Fig 1 Family tree of cases 1 5

and fruit, but more than half a pear will bring about violent nausea, vomiting, shaking and exhaustion lasting several hours. The symptoms are ameliorated by drinking milk and eating plain bread and butter

On examination the patient was found to be of normal build and height, slightly overweight and ith no hepatomegaly or dental caries. There was no proteinuria or glucosuria.

Determination of serum glucose, fructose phosphorus (Fig. .), FFA (Fig 6) and cholesterol were carried out.

Case 4 (E. Ar.), male, born at term 1894. Eight sibs of whom one has proved HFI (case 5) and two have ms of HFI (Fig. 1). The parents were healthy but paternal great grandfather could not tolerate sugar ("sugar tops make you sick to the death") and retained perfect teeth until his death at ninety The patient has four healthy children. He was breastfed, but does not know for how long. As very small infant he had been given honeycoated dummy but reacted with vomiting and pallor and soon learned to refuse it. Attempts to wean him with sweetened formula produced the characteristic vomiting and he refused this diet. The mother finally realized that he could not tolerate sugar and excluded it from his diet H then thrived and developed normally For as long as he can remember he too has had distaste for all sweets, fruit and certain vegetables, and attempts at eating such foods have resulted in severe vomiting and exhaustion lasting twelve hours. Apart from this reaction on ingestion of sugar the patient has always been healthy

On examination he was found to be of powerful build. There was no liver enlargement, dental caries, proteinuria or glucosuria.

The serum glucose, fructose, phosphorus (Fig. 5) and FFA (Fig. 6) were determined.

Case 5 (M. Ar.), male, born at term in 1903 Like his brother (case 4) he had vomited hen given sugared dummy or sweetened feeds. The mother, ho was now well conversant with the symptoms, immediately excluded sugar from his diet, and the infant thrived. He has had the same distaste as his brother for all sweets and responds in the same way to dietary errors.

Examination showed the patient to be of normal height and slight build. Like his brother his intelligence was normal. There was no hepatomegaly and the teeth were caries free. There was no glucosuria or proteinuria. During spring 1965 he developed glaucoma simplex.

Determination of glucose, fructose phosphorus (Fig. 3), FFA (Fig. 6) and glycerol (Fig. 7) were performed.

Cases 4 and 5 have two siblings ith symptoms characteristic of HFI, brother born in 1901 and living in Canada, and sister born in 1910. We have not examined them.

Case 6 (G S.), male born at term in 1930 with two healthy children. This patient's mother developed diabetes at 40 years of ge. There is no known consanguinity in this family and no cases with similar symptoms to the patient's are previously known in the family The patient was breastfed for three months, during hich time he thrived. Weaning to sweetened formula produced vomiting, pallor and malaise after each meal and by one month he had lost much weight. One morning he did not vomit after his feed and when the mother tasted it she found that she had forgotten to sweeten it. Thus it was discovered that the infant could not tolerate sugar, and the mother soon found out that the same applied to fruit All sweetened foods were subsequently withheld. As child he occasionally tasted sweets and fruit but always omitted afterwards, and became pale and shaky He soon developed distaste for all sweet food.

On examination he was found to be man of normal physical and mental development. There was no hepatomegaly caries, proteinuria or glucosuria.

The serum glucose, fructose, and phosphorus (Fig. 5) were determined. Bilirubin, thymol, alkaline phosphatase, GOT GPT and LD were also measured and found to be normal before and during the load.

Case 7 (I. N.), male, born at term in 1942. There are tw healthy siblings. The patient was breastfed for five months. Dietary supplements of fruit juice at three months inevitably produced vomiting, and the mother therefore stopped them. On ensuing sweetened feeds produced vomiting and the infant soon learned to refuse them, whereas he would accept plain milk. He could not tolerate fruit or most vegetables, which were therefore eliminated from the diet. No doctor was consulted. As a child he tried to eat sweets but developed nausea, vomiting and general weakness and malaise H felt better again if he ate crispbread with meat. During military service he was allowed special diet ithout medical examination and all ent ell until field training started. He was then unable to obtain suitable diet and his symptoms were interpreted as malingering.

The patient was a man of normal height, slight build and normal intelligence. There was no liver enlargement, caries, glucosuria or proteinuria.

Determinations of glucose, fructose, phosphorus (Fig. 5), FFA (Fig. 6) and glycerol (Fig. 7) were carried out.

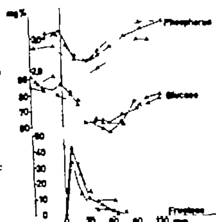


Fig 2. Concentration of phosphorous, glucose and fructose in blood after intravenous fructose tolerance test with 0.25 g/kg body weight in case 1 ( ), case 2 (——), and case 3 (. .).

RESULTS OF FRUCTOSE TOLERANCE TESTS

All HFI subjects complained of epigastric discomfort about ten minutes after injection of fructose. All reported a moderate sensation of hunger coming on about thirty minutes after the i jection. Two of four controls developed slight epigastric discomfort shortly after the injection. All HFI subjects showed a fall in blood glucose reaching a maximum 60–75 minutes after the injection of fructose (Figs. 2, 3 and 5). There was instead a rise in blood glucose among the controls and in the father of case 1 and 2 (Figs. 4 and 5).

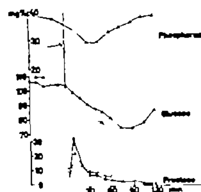


Fig. 3 Concentration of phosphorous, glucose and fructose in blood fter intravenous fructose tolerance test with 0.15 g/kg body eight in case 4 (- -) and case 5 (——).

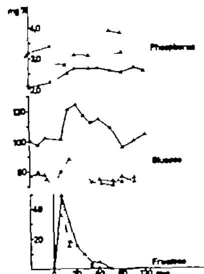
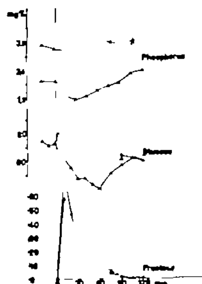


Fig 4 Concentration of phosphorous, glucose and fructose in blood after intravenous fructose tolerance test with 0.25 g/kg body weight in the father (ge 65) of cases 1 and 2 (——) and in two normal adult volunteers (aged 20 and 25)

The blood fructose level was roughly similar among subjects and controls, and was related to the size of the injection (Figs. 2–5). The urinary excretion of fructose amounted to about 10% of the dose among both subjects and controls.



F 5 Concentration of phosphorous, glucose and fructose in blood after intravenous fructose tolerance test th 0.35 g kg body eight in case 6 (. .) and in normal adult volunteer aged 1 (-), and th 0.25 g/kg in case 7 (——)

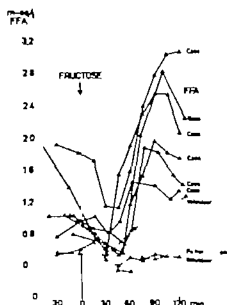


Fig 6 Free fatty acids in plasma during intravenous fructose tolerance tests in cases 1 5 and 7 (——) and 3 controls ( )

The serum phosphorus fell in all subjects by 0 8–2,4 mg/100 ml, with minimum 30–45 minutes after the fructose injection (Figs. 2, 3 and 5). The initial values had as a rule been regained after 90–120 minutes. Among the con- any fall was insignificant and briefly tran- ent (Figs. 4 and 5)

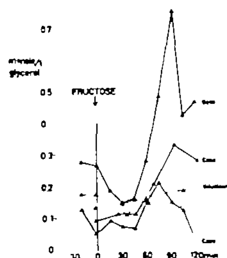


Fig 7 Plasma glycerol during intravenous fructose tolerance tests in cases _ 5 and 7 (——) and tw controls ( ).

In all subjects the FFA in plasma showed an initial fall followed by a rapid rise after 30-60 minutes, reaching a maximum after 75-90 minutes (Fig. 6) The controls showed an initial fall but the subsequent increase was less than among the subjects with HFI (Fig. 6).

Glycerol in plasma was determined in cases _, 5 and 7 and in two controls (Fig. 7). In case 5 and the controls the initial fall in FFA was accompanied by a corresponding fall in glycerol. In the other two subjects there was no such correlation between FFA and glycerol during the initial 30 minutes after the fructose injection. The subsequent rise in FFA showed a close correlation to glycerol in all subjects and the controls.

The serum cholesterol was investigated in cases 1 2 and 3 There was no significant change in the values.

Adrenaline in urine was determined in case 1, in a two-hour specimen before the fructose load and over a two-hour period during the test. The adrenaline excretion prior to the load was 0.4 *µg/hour and during the test 1 7 µg/hour*

DISCUSSION

Symptoms In all our subjects the symptoms characteristically appeared during the first year of life in association with weaning or when fruit juice was given. In two of them the symptoms developed at a few weeks of age, when they were given honey-coated dummies. All quickly developed aversion to sweet food-stuffs. This is the rule among children and adults although a few exceptions have been reported (10). Therefore, it is among infants, who cannot shield themselves against prolonged administration of fructose, that its sequelae—vomiting, failure to gain, severe attacks of hypoglycaemia, and liver and kidney damage—become manifest. *It is thus very important* to recognise the clinical picture so that fructose may be eliminated from the diet on a fructose-free diet these patients thrive, and develop normally All the subjects of this investigation were lucky enough to have observant mothers who quickly realized what their infants could not take, and eliminated it from their diet.

The fructose tolerance apparently increases with the years, and the patients come to be able to take small amounts. This was true of all our

subjects except cases 4 and 5 but it is difficult to assess whether these two had a stronger aversion to sweets or a severer degree of intolerance. Enzyme analyses on liver biopsy specimens might conceivably provide an answer. The possibility of different forms of HFI has been discussed, particularly by French workers (19–21, 36).

Physical findings. None of our subjects has shown enlargement of the liver, signs of renal damage, or mental retardation. Transaminase determinations were done in the course of fructose tolerance testing in case 6, but no increase was noted. As in previously published cases, all subjects of our investigation were free from caries, in contrast to unaffected members of the family. This illustrates the importance of sugar in regard to caries.

Fructosaemia. In the first cases of HFI to be published oral tolerance tests only, using high doses (1–2 g per kg body weight) were employed. High fructose levels (up to 160 mg/100 ml) were recorded, and the fructosaemia persisted for several hours.

Among controls, on the other hand, the fructosemia was insignificant (up to 30 mg/100 ml) and briefer (7, 11). Owing to the highly unpleasant gastro-intestinal symptoms resulting from oral administration, intravenous tolerance tests using smaller doses of fructose were introduced. The recommended dose is 0.25 g/kg body weight for adults, and 3 g/sq.m. body surface for children (13). These doses are sufficient to produce a fall in blood glucose and hypophosphataemia, whereas the fructosaemia remains moderate and does not differ significantly between subjects and controls. The degree of fructosaemia is thus not a diagnostic criterium in intravenous tolerance tests. Our results using varying doses of fructose indicate that the fructose level is probably related to the dose, and roughly similar in subjects and controls (Figs. 2–5).

Hypoglycaemia. To judge from our findings the time of maximum fall in blood glucose would appear to occur later with increasing age. Children develop hypoglycaemia more rapidly and on a smaller dose of fructose than do adults (13). The fall in blood glucose among our subjects was less than in certain reports in the literature. This is probably a question of dose. Case 6, where the dose was greater than in the rest of the series, reacted with a more marked fall in blood glucose in relation to the fasting level.

Hypophosphataemia. The fall in serum phosphorus preceded the hypoglycaemia in our subjects; the same has been described in the literature (13). According to Froesch, the hypophosphataemia is of greater diagnostic significance than the hypoglycaemia which may be significant in intravenous tests using small doses. Reports are given of falls in serum phosphorus of 0.5–2.5 mg/100 ml within 20–60 minutes, with restitution to normal after 60–150 minutes.

FFA, glycerol and adrenalin. In normal subjects the major part of the fructose administered is metabolized in the liver (24). Fructose can also be metabolized in normal adipose tissue by phosphorylation to fructose-6-phosphate (12, 23). In experiments in vitro Froesch & Ginsberg found that fructose in high concentrations is metabolized better in adipose tissue than is glucose. This is probably why in HFI 80–90% of the fructose administered is metabolized despite the deficiency of 1-phosphofructaldolase expecially in liver (13). It has been shown that fructose is metabolized to only a very slight degree in muscle (24, 33, 39).

In vivo glucose administration to normal subjects leads to increased re-esterification in the adipose tissue. This is shown by falling values for FFA and glycerol in plasma (?). Fructose probably has a similar effect in normal adipose tissue. On administration of fructose to our subjects with HFI a fall in FFA was noted, as among the controls, and one case showed a fall in glycerol during the first 30 minutes. In two of the subjects (cases 4 and 5) the fasting FFA were increased and fell before administration of fructose. This may well have been an expression of stress, as both individuals showed fear when the catheters were introduced. The fall in FFA would probably have been more pronounced if the method of Trout *et al.* (38) had been used instead of that of Dole (5), in which lactate and pyruvate are included in the determination to some extent. Cornblath *et al.* (4) has shown that an increase in lactate takes place within 30 minutes of intravenous administration of fructose in HFI.

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THE ROLE OF ALDOSTERONE IN HYPERNATREMIC DEHYDRATION IN INFANTS

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Acute hypernatremia is observed in infants and children mainly in the first two years of life. A disproportionate loss of sodium and water originating in acute diarrhea not balanced by appropriate intake is found in infants (7). Signs of moderate dehydration often are present. There is however a number of patients with severe symptoms of toxicity but only minimal fluid loss (7). The high mortality rate demonstrates the increased risk for these children. In the majority of cases the factors producing hypernatremia are well established.

The role of aldosterone in infants with hypernatremia or hypertonic dehydration has not been studied. There is general agreement that aldosterone secretion is influenced directly by acute changes in extracellular volume (2) Increased activity of aldosterone accompanies acute hypovolemia or is the result of a decreased catabolic rate in the liver

The following observation is the first report discussing the role of aldosterone in acute hypernatremia in infants.

CASE REPORT

Thomas, W This 11-month old boy was admitted to the Childrens Hospital for inguinal herniorrhaphy The infant is the first child of healthy parents delivered at term after uncomplicated pregnancy Besides a minor operation for phimosis the child has not been treated before

On admission the child was well developed and well nourished, body weight 11.4 kg, body length 77 cm. Herniorrhaphy was performed without complications on the 5th hospital day The night before operation the child vomited twice and had one soft stool This however was not considered of importance. The next morning the infant appeared well and operation was performed. On the same evening the temperature rose to 39.3°C, the child again had loose stools which soon became watery He was started on oral fluids and was given chloramphenicol by mouth. Despite this treatment symptoms of gastroenteritis persisted. Vomiting occasionally recurred although appetite was good. The child seemed thirsty and took all feedings well.

Two days after operation he obviously was dehydrated. He had suffered a weight loss of 10% of the body weight, his eyes were sunken, the skin was pale but turgor was normal The temperature continued to be elevated at 38.2°C. Serum electrolytes were at the upper limit of normal for sodium and chloride Hemoglobin had risen to 17.0 g % demonstrating a mild hemoconcentration. The child still was alert and took his feeding well. When vomiting increased parenteral fluid replacement was started. He was given a mixture of saline and 5% glucose/water with a relatively low sodium concentration (24 mEq/l).

With these measurements the infant had almost regained his weight loss the following day although he seemed to become unusually tired. Serum electrolytes now showed a marked hypernatremia and hyperchloremia with normal serum potassium. Blood pressure was 150/110 mm Hg, temperature 40°C. He also was observed to be oliguric The child's general condition deteriorated, he was drowsy but could be roused. Intravenous fluid replacement was continued using 5% glucose solution for one day followed by a low sodium solution (20 mEq/l) On the following day the temperature fell to normal, oliguria however persisted. The potassium level was 3.7 mEq/l, serum sodium and blood pressure remained elevated. During this period a short generalized convulsion occurred which was treated by intravenous mannitol 20%. A few hours later kidney function was restored with subsequent normalization of serum sodium and chloride concentrations Within three days all symptoms subsided, the child was started on oral feeding and tolerated the formula well. Serum electrolytes remained within normal limits, the blood pressure however was elevated for several more days before returning to normal.

When dehydration and oliguria were observed urine collection immediately was started The urine was analysed for aldosterone and electrolyte content. Aldosterone was determined by double isotope derivate assay (8), sodium and potassium by standard flame photometer chloride by Aminco-Cotlove chloridimeter

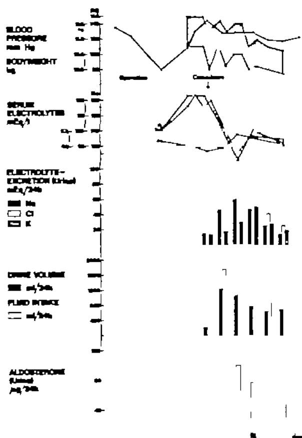


Fig. 1 Blood pressure, body weight, serum electrolytes, urine volume, electrolyte and aldosterone excretion.

1 gives the results of this series. When hypernat and oliguria developed excretion f electrolytes was minimal, aldosterone excretion however 97 μg/24 h. The following day polyuria started, sodium and chloride excretion increased reaching its maximum on the second day Chloride excretion 1 ays exceeded sodium probably due to higher chloride intake Potassium excretion also rose t 50 mEq day increasing with delay of 2 days however On the fourth day after polyuria had started electrolyte excretion returned to low level Aldosterone excretion declined gradually still being markedly elevated on the fourth day at 42 g 4 h. After polyuria had started there seemed to be no correlation between aldosterone excretion and electrolyte content of the urine

DISCUSSION

Most authors agree that several factors contribute to evolution of hypernatremia in infants (9 11 13). In the majority of cases water depletion without corresponding sodium loss results from dilute diarrheal stools. Darrow (4) and Weil & Wallace (15) have shown that sodium concentration can be as low as 19 mEq per liter of tool water Insensible water loss often is ncreased and water intake is reduced. Anorexia and vomiting further aggravate a negative water balance. Impaired renal function is a well known fact in dehydration in infants (3). Glomerular filtration rate and renal plasma flow are reduced leading to oliguria and nitrogen retention. A high solute load aggravated by catabolism and salt containing feeding requires a considerable fluid volume. Finally Skinner & Moll (13) have pointed out that salt excess is an important factor contributing to hypernatremia. Sodium intake exceeding 10 mEq/kg/day in infants treated for dehydration can not be handled by the kidney (14). Hypernatremia however also is known to originate in acute disturbances of the central nervous system, tubular insufficiency diabetes insipidus and osmotic diuresis.

The role of aldosterone in patients suffering from hypertonic dehydration has not been investigated as yet. This question however must assume great interest since alterations in aldosterone production can be anticipated. A decreased intravascular volume present in most states of dehydration immediately initiates a rising aldosterone secretion. This regulation attempts conservation of sodium and water at the same time The aforesaid mechanism has been shown independent of serum sodium concentration. Other factors are discussed as being important in regulating aldosterone secretion. Schwiegk (1) believes the intracellular electrolyte changes to be responsible for an increased aldosterone secretion. According to Gross (5) an aldosterone stimulating hormone (ASH) originates in the kindney It is part of the renin-angiotensin-system connecting closely kidney and adrenals. Furthermore aldosterone metabolism can be influenced by a decreased catabolism in the liver

All these facts will have to be taken into consideration in infants suffering from hypernatremia. In our case hypernatremia probably developed from disproportionate loss of water and sodium through watery stools. Salt intake never was excessive but might have been higher for 1– days than the relatively small sodium loss. The infant undoubtedly suffered acute dehydration w th fluid loss of 10% of body weight. In this situation hyperaldosteronism probably started as demonstrated in the high urinary aldosterone excretion. All ymptoms of hyperaldosteronism were present increased serum sodium level, low

potassium concentration, elevated blood pressure and low urinary excretion of sodium. Fluid deficit when discovered was overcome within one day all other symptoms however persisted. The continuously high aldosterone excretion suggests strongly an elevated aldosterone secretion although hypovolemia was subsided. This is in agreement with studies of Bartter By narrowing the carotid artery and reducing the pulse pressure he produced a prompt increase in aldosterone secretion. When normal blood flow was restored high aldosterone production persisted however It took two days until clinical improvement started and all symptoms disappeared quickly Aldosterone excretion fell too but did not normalize as fast as most other symptoms.

Very little is known about the role of aldosterone in hypernatremic states of infancy Whether aldosteronism in these infants is helpful in regulating deranged metabolism or exerts undesired effects, remains an open question. Derangements occurring in brain cells might account for cerebral symptoms.

In consequence, studies of aldosterone metabolism in infants with hypernatremia complicating dehydration will give valuable information on frequency and importance of high aldosterone secretion. If hypernatremia persists because of hyperaldosteronism therapeutic use of aldosterone blocking hormones such as spirolactone could be of great benefit. If however hypernatremia is at least primarily independent from aldosterone activity dialysis would be the alternative choice.

SUMMARY

In previously healthy 11-month-old infant acute gastroenteritis and dehydration developed following a minor operation. Oliguria was observed after 2 days complicated by hypernatremia, hypokalemia, elevated blood pressure and one generalized convulsion. Aldosterone excretion was increased considerably remaining elevated even after clinical improvement.

Hypernatremia complicating dehydration in infancy usually is thought to result from disproportionate loss of water and sodium. The role of aldosterone in this situation is not known. If hyperaldosteronism proves to be involved in causing and sustaining hypernatremia therapeutic use of aldosterone blocking hormones would be indicated.

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MALIGNANT PHEOCHROMOCYTOMA IN A CHILD TREATMENT WITH A COMBINATION OF ALPHA AND BETA ADRENERGIC BLOCKADE

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The symptomatic treatment of pheochromocytoma is directed towards pharmacological antagonism of circulating catecholamines. The medication must be adapted to the relative amounts of alpha and beta-adrenergic stimulating products from the tumor For several years, it has been possible to block alpha-adrenergic effects, either by means of competitive inhibitors like phentolamine or by means of "non-equilibrium inhibitors (2) like phenoxybenzamine These types of antagonists, however do not possess any beta-blocking activity Therefore the introduction of dichloroisoproterenol (4) and of its successors for clinical use, e.g. propranolol, greatly increased the therapeutic possibilities.

The present paper describes a case of malignant pheochromocytoma with hypertension which was first detected when the patient was 9 years of age The diagnosis was established by histopathological examinations as well as determinations of catecholamine levels in blood and urine It was found that alpha-adrenergic blockade with phentolamine was insufficient to control the symptoms but that a combination with propranolol gave a dramatic improvement of the patient's general condition. It is suggested that the use of both alpha- and beta-adrenergic inhibitors should be considered in cases of pheochromocytoma where radical surgery is not possible.

CASE HISTORY

I. J a girl born in October 1955 was admitted to this hospital in January 1966 She had an inoperable catecholamineproducing tumor in the lower pelvis with severe systemic hypertension. It had been detected in March 1964 at another hospital at examination because of abdominal pains. It was located in the right parametrium and was walnut-sized. In February and May 1965 new tumors appeared in the right groin and in the left parametrium. The histopathological picture following biopsy is shown in Fig. 1 The patient had attacks of headache with vomiting, palpitation, dyspnoea, and anxiety On one occasion pulmonary oedema had developed which necessitated digitalization. The blood pressure was reported to have been 180/120 mm Hg. A phentolamine test had been positive, and the 24-hour urinary excretion of adrenaline and noradrenaline had been found to be 34 μg and 238 μg, respectively Radical surgery had been attempted but found to be impossible because of the extensive invasiveness of the tumor Radiotherapy had been given postoperatively Phentolamine treatment (10 mg three times daily) was started in December 1965

SPECIAL INVESTIGATIONS

On admission to this hospital in January 1966 the girl was emaciated—body-weight in relation to length 8 kg below the average—and mentally depressed She had a constant fine tremor and outbreaks of sweating. No signs of tumor growth were found at palpation of the abdomen or the inguinal regions, nor at X-ray examination of the lungs and skeleton Physical examination, ECG and X-ray of the heart were indicative of cardiac enlargement and left ventricular hypertrophy The blood pressure in the supine position was elevated to around 180 170 mm Hg with occasional peaks up to 220 140 mm Hg. No hypertensive retinopathy was found. Over the second left interspace a systolic ejection murmur was heard and recorded Its intensity varied between grade 2 6 and—when the patient was anxious—grade 4 6 In the latter case it was high-frequent and stenotic in type The second heart sound was constantly split (0.04 sec) with easily audible components Thus auscultation indicated a certain prolongation of the right ventricular ejection time due to moderate outflow tract obstruction. The findings at right heart catheterization under general anesthesia were, however within normal limits. At angiocardiography considerable enlargement and severe hypertrophy of the left ventricle was demonstrated, which caused a

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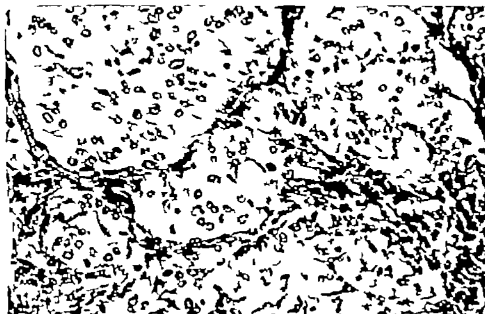


Fig. 1 Photomicrograph taken from lymph node metastasis removed in 1965. Sections re-examined by Dr. B. I. Iversen, Dept. of Pediatric Pathology. Tissue fixed in formalin and paraffin embedded. Only paraffin blocks were available for study and post-chromation of sections failed. Enlargement approx. ×100. Solid cords are seen of large, acinolated cells showing sparse stroma. In the lower right is seen a group of pigmented cells between the tumor cords. They represent deposits of melanin (dermatopathic lymphadenopathy) and form no part of the tumor. Diagnosis: Ectopic pheochromocytoma, originating from the organ of Zuckerkandl.

compression of the right ventricle. Routine blood and urinary analyses were normal. The urinary excretion of noradrenaline was strongly elevated. The pertinent data are summarized in Table 1.

Urological examination including radiographic studies did not permit any definite conclusions as to the localization and distribution of the neoplastic process. The same was true of determination of noradrenaline levels in blood from different parts of the inferior vena cava and its main contributaries (Fig. 2).

Table 1. *Summary of the clinical course*

	1966				1967	
	Jan. (First admission)	March	May	July	Jan.	Aug.
Body-length, cm	139				143	146
Body-weight, kg	21	24	26	29	31	34
Blood pressure, mm Hg	180/120	130/80	130/80	135/85	115/75	120/80
Heart rate/min	90	65	67	65	70	80
Heart volume ml/m² BSA	490		420	430	300	270
Urinary excretion per 24 h						
Adrenaline, µg	12			14	9	150
Noradrenaline, µg	214			112	216	318
5-HIAA, mg	5.5			8.8	10.9	14.0
VMA, mg	6.0			6.9	10.0	14.5
Treatment						
Phentolamine, mg/24 h	30	30	30	30	30	30
Digoxin, mg/24 h	0.25	0.25	0.25	—	—	—
Propranolol, mg/24 h	—	5→60	60	60→80	80	80

Mean values of six determinations during 4 hours with the patient continuously in the supine position.

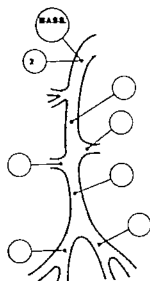


Fig Concentrations (μg/ml) of noradrenaline in blood from various levels of the inferior vena cava and its main contributaries. Blood samples were drawn in connection with right heart catheterization under general anaesthesia. The fluorimetric analyses were kindly performed by Dr A. Rosén. Adrenaline levels could not be accurately determined due t the xtremely high values for noradrenaline. Note that the renal veins have lower levels and that massage (MASS) over the inguinal regions did not increase the noradrenaline content in the blood.

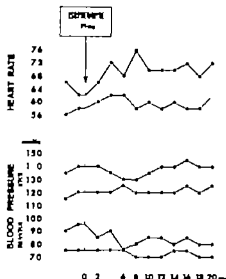


Fig 3 Test for the effectiveness f the beta-adrenergic blockade. Open symbols: Values obtained hen the patient had been without propranolol for two days; Closed symbols: Values obtained 4 hours after resumption of propranolol medication (80 mg/h). At both occasions, 15 mg of isoprenaline was administered sublingually at 0 min with the patient in the supine position. Placebo administration had no significant effect at either occasion. Note that 24 hours medication on propranolol resulted in decrease both in heart rate and blood pressure and that the effects of isoprenaline are stronger hen the beta-blocking agent has not been given.

TREATMENT

cal surgery as not possible but the radiotherapy ted in 1965 was completed Phentolamine treatment mg/mg three times daily) was continued In March, 1966, the beta-adrenergic blocking agent, propranolol, was added *to the phentolamine medication after previous digitalization*. Following digitalis, ventricular extrasystoles (VES), in frequency of 4–5 min were observed. Propranolol as first given in intravenous infusion (1 mg during 20 minutes) in order to test the patient tolerance to the drug This led to prompt disappearance of the VES but there ere no changes in heart rate or blood pressure Following this test, propranolol was given orally in doses gradually increasing to 20 mg four times daily

During this combined treatment, significant decrease in blood pressure gradually occurred dow to normal alues within three eeks (T ble 1) At the same time the general condition of the patient strikingly improved After another t weeks she was able to go back to school and to take part in all kinds of play There were no orthostatic symptoms.

In July 1966, the patient as readmitted to this hospital for evaluation of her condition A test was made for the effectiveness of the propranolol treatment by means of temporary discontinuation of the drug for three days under controlled conditions A slight rise in heart rate and blood pressure was noted already after t days. A simple test for sensitivity to beta-adrenergic stimulation was then made by giving the patient 15 mg isoprenaline sublingually and measuring heart rate and blood pressure (Fig 3) Propranolol treatment was then resumed in the *usual dose and after 4 hours of this medication* 15 mg isoprenaline was again given under the same conditions as on the day before It is seen in Fig. 3 that isoprenaline produced definite tachycardia and decrease of the diastolic pressure when propranolol was not given simultaneously However hen this beta-adrenergic inhibitor had been administered for 4 hours, no effect at all of isoprenaline could be noted, indicating full blockade to this dose of the beta-adrenergic stimulator Administration of placebo gav no effect.

During one and half years of medication the girl has been in excellent condition apart from slight urinary incontinence caused by the operation. She has increased normally in length and gained weight rapidly Digitalis medication was discontinued in July 1966. At a check-up in January 1967 she looked perfectly healthy in spite of the high urinary output of noradrenaline hich was elevated to the same extent as earlier The blood pressure was normal. She did not show any signs of puberty *The auscultatory precordial findings were normal* ith only faint and lowfrequent systolic murmur and normal second sound. The palpatory and ECG signs of left ventricular hypertrophy wer hardly significant, and the relativ heart volume wa normal (Table 1).

DISCUSSION

In a survey of the literature on malignant pheochromocytoma (3) it was pointed out that the total number of reports on this disease was small and that the diagnosis had been established only in a few of the published cases by means of catecholamine determinations. In spite of an increasing number of reports on pheochromocytoma in children the malignant form of the tumor is rare (1 9). In the present case, the malignancy was established histologically biochemically and at operation. The patient was only 9 years old when the tumor was first diagnosed

In a situation where radical surgery cannot be performed, a long-term symptomatic drug treatment must be instituted. The use of alpha-adrenergic blocking agents like phentolamine for this purpose is well established. Theoretically a concomitant inhibition of beta-adrenergic activity should be of value in those cases of pheochromocytoma where beta-adrenergic receptors are strongly stimulated. A hypotensive effect may then occur as a result of a block of the positive chronotropic and inotropic effects of the sympathetic nerves of the heart. Such a mechanism has been suggested to explain that chronic administration of pronethalol and of propranolol in hypertensive patients in general can lower the blood pressure without producing postural hypotension (5 6, 7).

From the theoretical point of view however a beta-blockade in pheochromocytoma may also be expected to produce an *increase* in the blood pressure if the tumor secretes predominantly *adrenaline* which possesses both alpha- and beta adrenergic effects. In that situation, the blockade might produce vasoconstriction by means of elimination of beta-adrenergic vasodilatation. In fact, such an increase of the blood pressure has been found in some patients (8).

In the present case, the *urinary analyses had* shown that noradrenaline was the dominating catecholamine secreted. Since circulating noradrenaline is a strong vasoconstrictor a beta-blockade on top of an alpha-blockade could possibly exert a hypotensive action by inhibition of the sympathetic influence on the heart. The dramatic effects of the propranolol treatment are in agreement with this interpretation.

It is obvious that the determina n of the relative concentrations of adrenal ne and nor adrenaline in the urine does not g enough guidance for the proper choice of drugs in the treatment of pheochromocytoma. P opranolol may well interfere with other mechanism for regulation of central and peripheral ci ul tion than with beta-adrenergic receptors. At th pres ent time, the precise mechanism for the h potensive effects of the drug is not fully unde tood (for references see (11)) It is obvious th t the combined treatment with alpha and beta adrenergic blocking agents was purely symptomatic because the clinical improvement occurred in spite of the fact that the tumor continuousl secreted high amounts of noradrenaline as see from the urinary analyses.

At the time when our patient was started on long-term medication with propranolol this drug had just been introduced in this country and no reports about its use for chronic treatment of pheochromocytoma were known to us. At the present time (Summer 1967) the patient has been on 60–80 mg propranolol per day for about one and a half years. During this time, no side effects at all have been observed. This confirms the general opinion that the chronic toxicity of propranolol is relatively low (10) As precaution, our patient was digitalized before the propranolol medication was instituted but digitalis could be early discontinued without any signs of cardiac failure

Even though the pharmacodynamics of beta adrenergic blocking agents are not known in detail, it is evident that they may be of great value in the symptomatic treatment of malignant pheochromocytoma also in cases where nor adrenaline secretion predominates over adrenaline secretion. *The dose should be gradually increased* until the hypertension is controlled. A beta-adrenergic blockade should be considered espe*cially in cases where* alpha-adrenergic blocking agents alone have proven to be insufficient. Recently Prichard & Ross (8) have reported about the usefulness of propranolol in conjunction with alpha-receptor blockade in controlling the symptoms of pheochromocytoma. It can be concluded from the present case that such a combined alpha- and beta-adrenergic blockade can be of great value even for chronic medication.

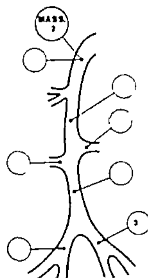


Fig Concentrations (g ml) of norad enaline in blood from arious levels of the inferior vena cav and its main contributaries. Blood samples ere draw in connec tion with right heart catheterization under general anaesthesia. The fluorimetric analyses were kindly performed by Dr A. Rosén. Adrenaline levels could not be accurately determined due t the extremely high values for noradrenaline. Note that the renal eins have lower level and that massage (MASS) over the inguinal regions did not increase the noradrenaline content in the blood.

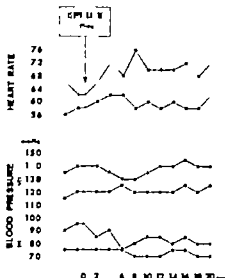


Fig 3 Test for the effectiveness of the beta-adrenergic blockade Open symbols: Values obtained hen the patient had been without propranolol for tw days. Closed symbols: Values obtained 4 hours after resumption of propranolol medication (80 mg h). At both occasions 15 mg of isoprenaline was administered sublingually at 0 min with the patient in the supine position. Placebo administration had no significant effect at either occasion. Note that 4 hours medication on propranolol resulted in decrease both in heart rate and blood pressure and that the effect of isoprenaline are stronger hen the beta-blocking agent has not been given.

TREATMENT

...ical surgery was not possible but the radiotherapy ...ed in 1965 was completed. Phentolamine treatment (10 mg three times daily) was continued. In March, 1966 the beta-adrenergic blocking agent, propranolol, was added to the phentolamine medication after previous digitalization Following digitalis, extrasystoles (VES), in frequency of 4–5 min ere observed Propranolol was first given in intravenous infusion (1 mg during 20 minutes) in order to test the patient tolerance to the drug. This led to prompt disappearance of the VES but there were no changes in heart rate or blood pressure. Following this test, propranolol as given orally in doses gradually increasing to 20 mg four times daily

During this combined treatment, significant decrease in blood pressure gradually occurred down t normal values within three weeks (Table 1) At the same time, the general condition of the patient strikingly improved. After another tw weeks she as able to go back to school and to take part in all kinds of play There were no orthostatic symptoms.

In July 1966, the patient was readmitted to this hospital for evaluation of her condition. A test was made for the effectiveness of the propranolol treatment by means of temporary discontinuation of the drug for three days under controlled conditions A slight rise in heart rate and blood pressure was noted already after two days. A simple test for sensitivity to beta-adrenergic stimulation as then made by giving the patient 15 mg isoprenaline sublingually and measuring heart rate and blood pressure (Fig. 3). Propranolol treatment was then resumed in the usual dose and after 4 hours of this medication 15 mg isoprenaline was again given under the same conditions as on the day before It is seen in Fig. 3 that isoprenaline produced definite tachycardia and a decrease of the diastolic pressure hen propranolol was not given simultaneously However hen this beta-adrenergic inhibitor had been administered for 4 hours, no effect at all of isoprenaline could be noted, indicating full blockade in this dose of the beta-adrenergic stimulator Administration of placebo ga no effect.

During one and half years of medication the girl has been in excellent condition apart from slight urinary incontinence caused by the operation. She has increased normally in length and gained weight rapidly. Digitalis medication was discontinued in July 1966. At a check-up in January 1967 she looked perfectly healthy in spite of the high urinary output of noradrenaline which was elevated to the same extent as earlier The blood pressure was normal She did not show any signs of puberty The auscultatory precordial findings were normal with only faint and lowfrequent systolic murmur and normal second sound. The palpatory and ECG signs of left ventricular hypertrophy were hardly significant, and the relative heart olume was normal (Table 1).

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DEVELOPMENT OF NON RESPIRATORY COMPONENTS OF ACID-BASE EQUILIBRIUM IN 250 NORMAL CHILDREN AGED ONE MONTH TO 15 YEARS

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The acid-base equilibrium is one of the main characteristics of the constancy of the internal environment. Changes of the acid-base balance are of importance both in the diagnosis and in the therapy, especially so in diseases of the respiratory system and in disturbances of intermediary metabolism. But routine determinations of acid-base balance were only made possible recently with the aid of exact and reliable micro-pH-meters and with the development of the micro-equilibration technique (1, 15) which only requires a sample of capillary blood. Leaving aside the question of how the composition of capillary blood reflects, under certain pathologic conditions, that of the actually circulating blood (5) another question remains open in pediatrics, e.g. whether the values accepted as normal for adults can be regarded as valid in childhood.

Comparatively large amounts of normal data are available in the postnatal period which is obviously due to the rising interest in the respiratory distress syndrome (9). While data of the development of acid-base balance from infancy up to adulthood are only sporadic (3) they seem to show that the acid-base balance differs from that in adults. Serial determinations of the complete acid-base balance in a sufficiently large number of normal children involve many technical difficulties. The purpose of the present paper has been to assess in the first place the easier accessible non-respiratory (metabolic) components of acid-base balance and their development from infancy to adulthood.

MATERIAL AND METHODS

Two hundred and fifty children aged one month to 15 years were examined in all. Out of them, 131 (52.4%) were boys and 119 (47.6) girls. All were healthy and normal children living in various institutions, i.e. three Infant Homes (infants up to one year of age), two Child Homes (one to three years of age) and one Recuperation Home (three to 15 years of age) for social reasons or because of protracted disease of the parents. Guided by previous experience we chose five age groups: group A comprised infants aged one to six months, group B infants aged six to twelve months, C children aged one to three years, D children aged three to nine years, E children aged nine to 15 years. In each group, 50 unselected subjects were examined. The sampling of blood was done in series of about 20 children at one sitting taking roughly one half from one and the other half from the neighbouring age group. The physician in charge of the children was consulted and all children suffering from any disease or those in convalescence were excluded.

Two to 3 ml of venous blood were sampled into dry heparinized test tubes which were kept stoppered in iced water up to the time of carrying out the analyses. After careful shaking the blood was transferred with the aid of Pasteur pipettes into the equilibration vessels of the Astrup micro-pH-meter (AME Radiometer) and, following three-minute equilibration with known CO_2 tension, the pH was determined (15). The volume per cent of CO_2 in the bottles was determined, before each examination series, by the Scholander method (11) and the CO_2 tension corrected for the actual barometric tension which varied, in individual sampling series, from 735 to 750 mm Hg. Standard bicarbonate, buffer base, and base excess were then read in the usual way on the Siggaard-Andersen nomogram (12). To enable later comparison of the slope of the equilibration CO_2 curves, the pH of each equilibrated blood sample was read also for P_{CO_2} corresponding to exactly 30 and 60 mm Hg.

Hemoglobin concentration in the sampled blood was determined photocolorimetrically as cyanmethemoglobin (17).

The blood samples were then centrifuged and specific gravity of plasma was determined with the aid of gradient cylinder; from this value the concentration of total plasma protein was calculated using the formula given by the authors of the method (8).

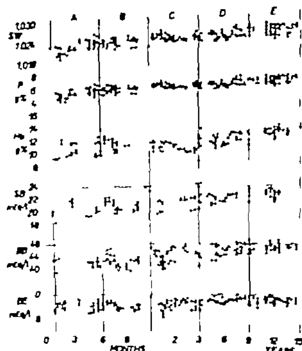


Fig 1 Distribution of individual values in individual age groups A to E *SW* specific gravity of plasma *P* total protein concentration in g per 100 ml plasma *Hb* hemoglobin concentration in g per 100 ml blood *SB* standard bicarbonate in mEq per liter plasma *BB* buffer base in mEq per liter blood *BE* base excess in mEq per liter plasma. Interrupted lines in the last three values give normal levels for adults.

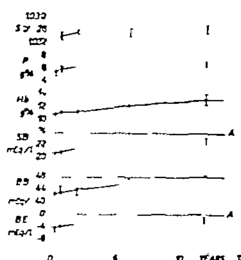


Fig Mean values in individual age groups and their standard deviations. Designation of the ordinata is the same as in Fig. 1 Interrupted lines (*A*) give normal values for adults.

RESULTS

All values determined or calculated in the whole group of 250 children are shown in Fig. 1 The time axis of this figure has been chosen so that equal lengths correspond to each of the five age groups. It is clear that, in the individual age groups, the distribution of the examined children is practically ideal the values covering evenly the entire chosen age span. The rising trend of all values with age and the dispersion of values is very evident.

Mean values and standard deviations in each age group are presented in Fig. 2 which has the normal linear time axis. The numeric values of these means, of standard deviations, and the minimal and maximal values are shown in Tables *1 and 2 The lowest values can be found in the* youngest age groups with increasing age they come near the normal adult levels. But the concentrations of standard bicarbonate, buffer base, and base excess never reach, even in the oldest *children (9 to 15 years), the normal adult values.*

Fig. 3 shows that the mean CO_2 equilibration curves have, in the youngest age groups, a less steep slope than that presumed in normal blood in the

Table 1 *Mean values, their standard deviations* (S.D.) *and minimal and maximal values (range) of specific gravity of plasma, of total plasma protein (P) and of hemoglobin (Hb) concentrations in individual age groups*

Age group	Specific weight of plasma Mean	S.D.	Range	Plasma total protein (g/100 ml) Mean	S.D.	Range	Hemoglobin (g/100 ml blood) Mean	S.D.	Range
A, 1–6 months	1 02320	0.00184	1 0195–1 0271	5 66	0.67	4 4–7.0	10.97	1 03	8 6–13.4
B, 6–12 months	1 02448	0.00117	1.02_2 1 0266	6.08	0.41	5.3–6.8	11 30	1 19	8 9–13.7
C, 1 3 years	1.02585	0.00083	1 0244–1 0278	6.55	0.31	6.1 7_	11.34	1.27	8.8–14 3
D, 3–9 years	1 02591	0 00116	1.0242 1 0290	6.58	0.40	6.0–7 7	12.45	1 09	9 8–14.9
E, 9–15 years	1 02711	0.00124	1 0?42 1.0?97	6.99	0.43	6.0–7 9	13.38	0.84	11 4–15.5

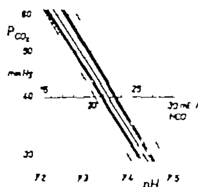


Fig. 3. log P_{CO_2}—pH diagram of CO_2 equilibration curves of blood. Interrupted lines give normal slope of curves with 20 and 25 mEq/l standard bicarbonate concentration. Full lines stand for the found mean slope in individual age groups A to E (left to right).

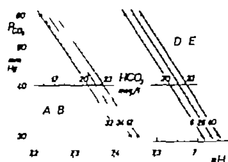


Fig. 4. log P_{CO_2}—pH diagram of CO equilibration curves of blood. Full lines represent the found equilibration curves of blood with an average standard bicarbonate of 20.4, 21.4, and 22.4 mEq/l. Interrupted lines represent the presumed slope in blood of normal adults with corresponding standard bicarbonate values. Curves of infants aged one to 12 months (age groups A and B) are in the left part of the figure, those of children aged three to 15 years (age group D and E) are in the right part. N gives the number of blood samples examined for each average standard bicarbonate concentration.

nomogram for adults. But let us not forget that the mean equilibration curves for each age group have been calculated from all the individual curves which had widely varying concentrations of standard bicarbonate. To be able to better understand this finding we calculated the mean equilibration curves for concentrations of standard bicarbonate equaling 20.0 to 20.9, 21.0 to 21.9 and 22.0 to 22.9 mEq/l together for the two youngest age groups A and B and the two oldest age groups D and E. The groups A and B and D and E could be fused on account of the practical identity of both the slope and the position of their mean equilibration curves (Fig. 3). Fig. 4 shows that the slope of blood CO equilibration curves in children aged three to 15 years (Group D and E) is the same as the presumed slope of the blood of normal adults possessing the same concentration of standard bicarbonate.

In contrast to that, the equilibration curves in infants from one to 12 months of age (group A and B) have a less steep slope than the presumed adult curves. The blood of infants thus has a lower buffering capacity than the blood of older children or of adults possessing the same concentration of standard bicarbonate.

COMMENTS

As has already been mentioned, the blood was sampled always in about 20 children belonging, in turns, to two neighbouring age groups. In the first phase of our work 150 children from all five age groups were examined during March and April 1965. The remaining 100 children were

Table 2. *Table 1 continued. Mean values, standard deviations* (S.D.) *and minimal and maximal values (range) of standard bicarbonate, buffer base and base excess in the same children*

There were 50 children in each age group

Age group	Standard bicarbonate (mEq/l) Mean		Range	Buffer base (mEq/l) Mean	D.	Range	Base excess (mEq/l) Mean		Range	
A, 1–6 months	20.77	1 14	18 4–22.8	42.76	1 85	38 0–47 2	4 06	1 3	7 1 to	1 4
B, 6–12 months	20 99	1.28	18.0–24 0	43 03	2.13	39 3–47 9	3.77	1 70	7 9 to	0.0
C, 1 3 years	21.38	1.21	18.6–24.3	44 05	1 90	40 1–48.0	3 00	1 58	7 1 to	0 4
D 3–9 years	22 24	0.96	20.3–24 4	45.88	1 75	41.2–49 1	2.11	1 4	4.8 to	0 6
E, 9–15 years	22.44	0.76	20.5–23 7	46.64	1 76	42.3–49 1	1 88	1 05	4 9 to	0.2

examined in the same manner during November of the same year. These latter results did not differ from those gained in the spring months. This approach warranted that eventual systematic errors of the applied methods were the same in the entire examined material and that results in the various age groups were strictly comparable.

Several formulae are known for the calculation of the concentration of plasma protein from specific gravity of the plasma. The original formula by the authors of the gradient method (8) as used by us gives results which are by 0.2 to 0.4 gm per 100 ml of plasma lower than the results by other formulae (16). While there is some uncertainty with regard to the absolute values of the concentration of total plasma protein the gravimetric determination is sufficiently reliable to demonstrate the development of the level of plasma protein during childhood, especially so as only normal children were examined. The low values in infants and the increase with increasing age had been assessed by other methods (6, 10) as well. Plasma protein being a component of the reaction dependent anions of plasma the fall of its concentration in infants leads to decrease of the buffering strength of plasma.

Data in the literature on the concentration of hemoglobin in childhood greatly differ (4). This is due to a certain measure to the technique of determination and the used standards. The cyanmethemoglobin method used by us, while regarded as one of the most exact, seems to give results by about 4 to 5% lower than the real ones (17). In spite of that it is clear that in infants and children up to three years the hemoglobin concentration is significantly lower than in adolescents and adults. Hemoglobin is an important component of the buffering system of blood and it is to be expected that a decrease of its concentration will be reflected in the decrease of the buffering strength of whole blood.

The low CO_2 combining power (T 40) of the blood in normal children above two years of age was found already in 1953 by Cassels & Morse (3). Their results converted to values of standard bicarbonate correspond to our results in the respective age groups C to E, similarly as the findings of Boda & Murányi (2). The youngest age group (our A and B) seems to have been neglected until recently when two papers appeared giving negative base excess (18) and decreased standard bicarbonate (19) closely corresponding to our findings.

The standard bicarbonate concentration in children, while increasing with increasing age, does not show any tendency to reach normal adult values (Fig. 2). The concentration of 24 mEq/l accepted by Siggaard-Andersen as normal for adults seems to be somewhat higher than the real value (7).

Buffer base and base excess were read from the nomogram for adults (1…) as there is no special nomogram for children. The nomogram is constructed under the presumption that the hemoglobin concentration equals 15 g per 100 ml of blood and total protein 7.3 g per 100 ml of plasma. Fig. 4 shows clearly that, in infants up to one year of life having an obviously lower concentration of hemoglobin and lower plasma protein, the slope of the equilibration curve does not correspond to the presumed slope in the nomogram.

The slope of the equilibration curve reflects the non-bicarbonate buffer system of blood (14); the markedly lower hemoglobin concentration as found in infants and young children makes itself felt in the first place. The simultaneous fall of the concentration of plasma protein has not such a great influence because plasma protein only forms about a sixth part of the non-bicarbonate buffer system. The fall of hemoglobin concentration in infancy represents about a third (about 2 mEq/l) of the found decrease of buffer base; it seems therefore that, next to standard bicarbonate, base excess is more suitable for the evaluation of the metabolic component proper of acid-base balance than buffer base. For clinical purposes it is not necessary to correct the found base excess for the decreased hemoglobin concentration because, when comparing it to the corrected values read in the Siggaard Andersen alignment nomogram (13), it can be seen that the difference is very small and, with regard to the dispersion of individual values, negligible.

CONCLUSIONS

The ontogenetic development of the metabolic components of acid-base balance has a characteristic increasing trend. In infants as well as in young children up to the third year of life a marked decrease of both standard bicarbonate

concentration and buffer base are found, implying that the base excess is negative and the buffering capacity of blood somewhat lowered. There seems to be, at that period of life, a certain tendency towards non-respiratory (metabolic) acidosis which is corrected and normalised with increasing age. These signs of non-respiratory acidosis cannot be regarded as pathologic because they have been found repeatedly and regularly in completely healthy and normal children. On the other hand, the dispersion between minimal and maximal individual normal values is so large that while examining a sick child for the first time the assessed value of the metabolic component need not be a reliable diagnostic criterion and only after repeated examinations in the course of illness and treatment can it be said whether that value had, or had not, been pathologic at the beginning.

SUMMARY

Total plasma protein, hemoglobin, standard bicarbonate, buffer base, and base excess concentrations were determined in venous blood of ..50 healthy normal children of both sexes aged one month to 15 years. In infants up to one year (100 babies in all) the mean standard bicarbonate concentration was round 1 mEq/l, buffer base 43 mEq/l, and base excess −4 mEq/l. A slight lowering of the buffering capacity of blood due, above all, to decreased hemoglobin concentration was found in these infants.

With increasing age all values come near the normal adult values but do not quite reach them even in the oldest age group of children aged 9 to 15 years. Infants especially but also children from one to three years of age have, in comparison to adults, a marked trend towards non-respiratory (metabolic) acidosis that, however cannot be regarded as pathologic.

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REVIEW ARTICLE

CRANIOSYNOSTOSIS

Review of the Literature and Indications for Surgery

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Since 1851 when Virchow (58) coined the term, craniostenosis has attracted the interest of different categories of doctors, such as pediatricians, ophtalmologists, neurosurgeons, pathologists. This has been documented in umerous case reports, review articles and monographs. Although most aspects of the disease are well documented in large series (2–4 9–11 19 25 28 29 35 38–45 48 51), there is no general agreement regarding the indications for surgery This disagreement has prompted us to an effort to clarify the situation in light of our own experience together with a review of the literature.

In order to understand the current problems of treatment it is necessary to know the history of craniostenosis and the evolution of therapeutic concepts during the years.

HISTORY

Sömmering (54) is considered *to be the first to* carry out scientific investigation on cranial deformities. His theories were further developed by Otto (47), but it was Virchow who finally in 1851 (58) proposed the classic theory known as Virchow s law This states that when premature fusion of the cranial vault occurs there is an nhibition of the normal growth of the skull in direction perpendicular to the suture which is fused, which gives a compensatory growth in direction parallel to the fused sut re. Virchow believed that such a deformed cranium obstructed the normal growth of the bra and accordingly he named the disease craniostenosis, which means a narrowed or strictured skull. Sear instead recommended adoption of the term craniosynostosis in order to include all varieties of premature closure of the cranial sutures (50) The idea that normal cerebral growth was impaired resulting in signs and symptoms of increased intracranial pressure was soon generally accepted. von Graefe (22) has been credited for recognition of visual impairment secondary to skull deformity During the following decades many papers were published unfortunately with a growing tendency to confuse craniosynostosis with microcephaly probably due to lacking the possibil ty of x-ray confirmation of the diagnosis. Thus, when Lannelongue (37) and Lane (36) started to operate upon craniostenosis with decompressive linear craniectomy in order to relieve the symptoms many of the patients were not craniosynostotic but microcephalic. Accordingly the results were not overwhelming and when Jacobi (31) reported 33 operations of mixed series w th bad results *and high mortal ty t meant the end of cranio*synostosis surgery for nearly 30 years, until Faber & Tow in 1927 (16) proposed the revival of surgery in order to prevent blindness and other complications. 1943 the same authors (17) stressed the importance of early operations and uggested the optimal time as 1–3 months age, when the operative risk is reasonably low and the prospect of satisfying results is best. This t me schedule was generally accepted. However it was not generally accepted that a craniectomy should be performed at all mainly because of many unsatisfying operative results due to rapid bridging of the artificial sut res w th new formed bone-bridges. This problem was mainly overcome by

Simmons & Peyton, who in 1947 (5) suggested covering of the margins of the bone defect with tantalum foil in order to retardate bony reunion. They also stressed that the probability of preventing mental retardation is the most important indication for surgical intervention. Since that time many technical improvements have been suggested by for instance Ingraham *et al.* (28–30), McLaurin & Matson (45), Anderson & Johnson (5), Wiegand (59) Mullan (46) Klein (34) Sorour (53), Teng (56) Anderson & Geiger (3), Powiertowsky & Matlosz (49) Large series treated with linear craniectomy have been published and the tendency during "the fifties was to operate upon all cases of craniosynostosis without any selection, mainly because of the high incidence of mental retardation and intracranial pressure symptoms reported by Larsen (42), Brav (14) Günther ( ) Bertelsen (1, 13)

During the last years, however the advisability of profylactic craniectomy in all cases of craniosynostosis has been seriously questioned by for instance Hemple *et al* (25) and Freeman & Borkowf (19). They have questioned the advocation of craniectomy for cosmetic reasons alone because if craniectomy is proposed to relieve or prevent the intracranial pressure resulting from reduced capacity of the skull, the evidence that such increased pressure actually exists or may reasonably be expected to develop should be quite ... lte. There is no general agreement that such evidence is present or predictable. This appears to be specially true for craniosynostosis, involving the sagittal suture only" (25).

The combined results of Hemple *et al* and Freeman & Borkowf presents 37 cases with isolated sagittal suture fusion and mental retardation in 5 of them (19). They could not find a detectable difference in mental or cosmetic results between the operated and unoperated cases, but this was when compared with the total series of all varieties of synostosis. Ingraham *et al.* (28) reported 40 % mental retardation in a series of 50 patients, but in 20 patients receiving primary surgical treatment before the age of one year only one was definitely retarded. Observations regarding high incidence of mental retardations in older patients suffering from craniosynostosis are reported in other series with similar results (2, 3 38–41 45 52).

Shillito & Matson (51) "feel that probably optimum development and function of the brain is permitted by providing it the chance to assume its natural shape and rate of expansion as soon as possible" They also consider the cosmetic indication rather strong. Every individual should have an opportunity to get a normal appearance of the skull. The same opinion is adopted by Anderson & Geiger (3) and they justify this free approach with the simplicity of the surgical procedure and the low risk of complications.

CASE MATERIAL

The present study includes 38 patients with craniosynostosis from the Neurosurgical Department of the Sahlgren Hospital 1954–66. The cases with iatrogenic synostosis after shunting procedures are excluded (6). They were classified into 8 groups.

I. *Simple craniosynostosis.*

A. Scaphocephaly—premature fusion of the sagittal suture (8).
B. Brachycephaly—premature fusion of the coronal suture.
C. Oxycephaly—premature fusion of the coronal and another suture
D Plagiocephaly—premature fusion of coronal suture on one side.
E. Trigonocephaly—premature fusion of the metopic suture.

II *Craniosynostosis with added anomalies.*

A. Crouzon disease (dysostosis craniofacialis—exophthalmos, small maxilla, prognathism, beaked nose and premature fusion of any or all cranial sutures).
B Apert syndrome (acrocephalosyndactylia—premature fusion of any type, associated with syndactylia).
C. Carpenter syndrome (acrocephalopolysyndactylia—acrocephaly peculiar facies, brachysyndactyly of the fingers, preaxial polydactyly and syndactyly of the toes, hypogenitalism, obesity mental retardation). Additional features may be coxa valga, genu valgum, pes varus, congenital heart disease and abdominal hernia (7, 55).

The subdivision of our cases, sex distribution, principal symptoms, eye findings, X-ray findings and mental symptoms are summarized in Table 1. 71 % of the cases were admitted during the last three years. Linear craniectomy following Ingraham (28–30) or Teng (56) technique was performed in 36 cases There was no operative mortality and the post-operative complications were few and of minor degree 6 patients had to be reoperated upon because of bone bridges over the craniectomy gap. The timing of operation is scheduled in Fig 1. The distribution and findings are all in accordance with larger published series, but one case, which is really interesting and of utmost importance in discussing the indications for surgery will be described in detail.

Table I. 38 Cases of craniosynostosis

Suture involved and type of skull deformity	No.	Sex M	Sex F	Family history		Principal signs and symptoms (except eye findings)		Positive eye findings		X ray findings		Mental status	
Sagittal	16	15	1	Pos.	3	Deformity only	12	Papilledema	2	Plain X-ray:		Normal	14
				Neg.	11	Increased ic. pressure	2	Strabismus	1	Increased ic. pressure	3	Retarded	2
Scaphocephaly	(42.1 %)			No inf.	2	Seizures	1	Hyperopia	1	Encephalography:	5		
						Jejunal atresia	1			Normal	3		
						Aqueductal stenosis	1			Cerebral atrophy	1		
										Aqueductal stenosis with hydrocephalus	1		
Coronal unilateral	2	1	1	Pos.	0	Deformity only	1	Strabismus	1	Plain X-ray:		Normal	1
				Neg.	2	Unilateral syndactyly of the III and IV toes	1	Hypertelorism	2	Increased ic. pressure	0	Ret.	1
Plagiocephaly	(5.3 %)							Amblyopia (normal fundi)	1	Encephalography	0		
Coronal bilateral Brachycephaly	0												
Metopic suture	6	3	3	Pos.	0	Deformity only	5			Plain X-ray:		Normal	5
				Neg.	3	Mongoloid face, omphalocele, partial Marfan's syndrome and Fallot tetrade	1			Increased ic. pressure	0	Ret.	1
Trigonocephaly	(15.8 %)			No inf.	3					Encephalography:	1		
										Widening of cisterna veli interpositi	1		
Mixed (two or more sutures)	7	4	3	Pos.	4	Deformity only	3	Papilledema	3	Plain X-ray:		Normal	7
				Neg.	2	Increased ic. pressure	3	Hypertelorism	2	Increased ic. pressure	5	Ret.	0
Oxycephaly	(18.4 %)			No inf.	1	Cleft palate, luxation of the elbow and knee joints, bilateral club-fot and partial Marfan syndrome	1	Epicantus	1	Encephalography:	4		
								Abducens palsy	2	Normal	2		
								Ptosis	2	Deformation of the ventricular system due to skull deformity	2		
Crouzon syndrome	4	4	0	Pos.	1	Deformation only	3	Exophthalmos	4	Plain X-ray:		Normal	4
	(10.5 %)			Neg.	1	Total atresia of the external auditory canal	1	Papilledema	1	Increased ic. pressure	1	Ret.	0
				No inf.	2			Strabismus	1	Encephalography:	2		
								Hypertelorism	2	Hydrocephalus	1		
								Decreased vision	1	Deformation of the ventricular system due to skull deformity	1		
Apert's syndrome	2	1	1	Pos.	0	Deformation only	0	Exophthalmos	1	Plain X-ray:		Normal	1
	(5.3 %)			Neg.	2	Cleft palate	1	Papilledema	1	Increased ic. pressure	1	Ret.	1
						Hypoplastic maxilla	1			Encephalography	1		
										Deformation of the ventricular system due to skull deformity	1		
Carpenter syndrome	1	0	1			Deformation only	1	Hypertelorism	1	No signs of increased ic pressure. Encephalography with normal findings		Retarded	
	(2.6 %)			Neg.	1			Epicantus	1				

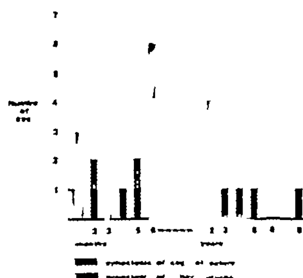


Fig. 1. Age at operation.



Fig. 3.

CASE REPORT

A 9-year-old boy, two elder siblings both normal. No heredity for cranial deformities. At one year of age the boy had pharyngitis with convulsions and high fever. A cranial X-ray was done and it showed scaphocephalic head with premature closure of the sagittal suture. Small and faint digital markings in the parietal bones (Figs. and 3).

Nothing important happened during early childhood. The boy started school at the usual time and was considered normal, except for a long narrow head and some difficulties in learning. When he was 8 years old he started having headaches of pressure type, mostly early in the morning disappearing later in the day. There had been no trouble before with his vision, but now he was sent to the ophthalmologist, who diagnosed the visual acuity to 0.9 bilateral. The boy's teacher noticed that the boy was slowing down in his psychic development. He was hospitalized for investigation of his scaphocephalic deformity. X-ray of the skull showed: high-grade scaphocephaly with complete closure of the sagittal suture



Fig. 2.



Fig. 4.

All other sutures were open. The digital impressions in the skull were fairly prominent but there was no excavation of the sella (Fig. 4). A new visual test showed right e) 0.7 left eye 0.8 and there was incipient papill-edema bilaterally. Echo-encephalography showed high amplitude and forceful pulsations as has been reported with high intracranial pressure (32). Pneumo-encephalography and EEG were quite normal. The child was operated upon with parasagittal craniectomy using the technique of Tong (56). The postoperative course was uneventful.

The child was examined 1 / years after the operation and it as found that the head circumference had increased 1 cm. His headache had completely vanished and his visual acuity had returned to 1.0 bilaterally. There were no more signs of intracranial pressure in the fundi and his school performances had definitely improved. The parents were very satisfied with the result of the operation.

DISCUSSION

The etiological factors involved in craniosynostosis are still unknown, but it is generally accepted that the major clinical manifestations are due to the premature fusion of the cranial vault sutures, which has made it logical to direct the therapy towards creation of artificial sutures. If a craniectomy should be done for prophylactic reasons one must, however accept that a premature closure of the cranial sutures will restrict the skull capacity and disturb the normal expansion of the brain during its actual growth. Assessment of the skull capacity is very difficult and so far no estimations of the true skull capacity in living subjects are available. Thus, most of our conceptions on the subject must be based upon accumulated clinical experience. The most important factors are probably the specific sutures involved and the time of their closure correlated to the patient's age.

There are two main indications for carrying our prophylactic operative treatment.

1 Prevention of the effect resulting from constriction or distorsion of the growing brain.

– Prevention of the cosmetic deformity

When 2 or more sutures are involved, the indications for surgery seems to be absolute, depending on the fact that most of these patients will sooner or later develop increased intracranial pressure with ocular symptoms, which might require urgent surgical attention (1–3 1– 13 19 20 25 27–29 38–41 43–46, 48 51 5– 57). Cases of craniosynostosis published around 1900 were mostly reported by *ophthalmologists* consulted by blind craniosynostotic pa nts. They were usually young or adult persons th brachy*cephalic deformities. It is possible that* besides the increased intracranial pressure other factors may be involved in these cases, such as the direct influence of bone abnormalities upon the optic nerve (23). However the results obtained by decompressive operations upon papilledema makes it probable that the increased intracranial pressure is the most important factor involved. Laitinen *et al* (38–41) and Bertelsen (13) have reported that cerebrospinal fluid pressure returns to normal values after decompressive craniectomy in cases with craniosynostosis.

The decision whether to operate upon an isolated synostotic suture is much more complicated In an attempt to clarify the situation we made a review of the literature with intention to collect cases where intracranial pressure and mental retardation could be ascribed to an isolated suture fusion. The key to the problem must be to find the exact figures of mental retardation and intracranial high pressure in untreated cases, because these complications of the disease are of main importance for the patient's welfare. Altogether we were able to collect 19 cases, where ocular disabilities probably due to intracranial pressure could be ascribed to an isolated sagittal suture fusion (3 15 24 26, 39). In cases described in the 19th century the diagnosis is sometimes doubtful as for instance in the classical case, described by von Graefe (22). The collected evidence makes it still reasonable to postulate that the potential risk for increased intracranial pressure is a definite risk, even if it is small one. It was neither possible to evaluate the exact percentage of cases, where intracranial high pressure develops nor to find a diagnostic clue to which cases increased pressure can be expected. We have also found that it is nearly impossible to get reliable figures upon the incidence of mental retardation in these cases. It is observed in most series that some cases of isolated suture synostosis have mental retardation, but mostly it is impossible to tell if the mental retardation is primary or if it is secondary to craniosynostosis. W were not able to find large material where there has been comparison between an untreated and a treated series of craniosynostosis with special reference to mental retardation It has not been proved that mental *retardation is always consequence of cranio-*

synostosis, although it is probable. We have not been able to find any convincing evidence in favour of the statement of Gordon (21) that mental retardation may be associated with but not a result of craniosynostosis. Deformation of the brain due to the cranial deformity probably does not cause severe malfunction even if it is reported that mental retardation is more common in cases with fusion of the metopic suture than in most other types of craniosynostosis (4). Regarding this special type of fusion the incidence of other congenital malformations is also significantly higher and no cases have been reported with increased intracranial pressure and fused metopic suture. Operation does not relieve all forms of mental defects accompanying craniosynostosis especially not if long-standing chronic intracranial pressure has caused irreparable brain damage On the other hand it has definitely been shown that improvement can be obtained by decompressive craniectomy

The particular case we have reported is interesting because the patient shows signs of increased intracranial pressure, which developed relatively late in the history of a craniosynostosis, and at the same time he showed psychic retardation which vanished after craniectomy

In favour of early operation for cosmetic reasons, cases are reported, where the increasing deformity of the skull has forced the surgeon to when the child has grown up (46) e surgical problems are in these cases much more complicated and the extensive operations required (33 46, 49) are apt to give both higher mortality and morbidity

CONCLUSIONS

We would like to emphasize the collected evidence in favour of early operation of all cases of craniosynostosis.

1 High incidence of mental retardation in the group of multiple synostosis due to high probability for increased intracranial pressure and impairment of normal growth of the brain.

2. Marked improvement after decompressive craniectomy observed in some cases with behaviour problems.

3 Increased incidence of mental defects in older patients with untreated craniosynostosis. Significantly lower incidence of mental retardation in patients operated upon early compared with those operated late.

4 Reported cases with evidence of raised intracranial pressure with only one fused suture.

5 The simplicity of the surgical procedure if it is done during the first months of life.

This collected evidence and the experience from our own series have satisfied us that cases with craniosynostosis should be operated upon as early as possible in order to prevent brain damage in some of the cases. Which these cases are is not predictable during the optimal time for surgery Secondarily we also believe that the importance of the cosmetic appearance should not be minimized.

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CASE REPORT

THYROID CRISIS IN A 3-YEAR-OLD GIRL

Inge Lise Dahl

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Thyrotoxicosis in childhood is relatively unusual. According to large surveys of child thyrotoxicosis (4 9 18) about 60–80 % of those who have the disease are in prepuberty or puberty 15–27 % are from 6 to 9 years of age, and 5–10 % are less than 5 years of age. The disease is 6 to 7 times more frequent in girls than in boys.

Thyroid crisis in children is an uncommon disease. The available literature reports it only in connexion with thyrotoxic mothers who deliver children with thyroid crisis (10, 13 16), and "apathetic" thyrotoxicosis with resulting crisis condition in a 10-year-old girl (2).

CASE REPORT

Girl, 3 years old, normally developed and earlier healthy The mother had colloid goiter since puberty Otherwise, nothing notable in the heredity Pregnancy and partus normal. Birth weight 3380 g. Since she was 12 months old, the girl hade diffuse enlargement of the thyroid gland and slightly protuberant eyes During the past year her exophthalmus had worsened, and she had become more restless, nervous and tired. The last months prior to admittance, she had, periodically tremors in the body and excessive perspiration during the night, and was generally troubled by heat. Despite ravenous appetite, her eight was not appreciably increased, although her growth in length was considerable. The stools were frequent, but not loose. After having been quite well the day before, she was admitted to the Childrens Hospital in Halsingborg on the morning of August 5th, 1966 in soporous condition, pale, with slight general cyanosis and with rapid superficial respiration She had considerably reduced tissue turgor dripped with perspiration, had striking exophthalmus, temperature of 40 5°C, tachycardia of 220 beats per minute and systolic murmur over the entire heart. Blood pressure was 170/70 mm Hg. The consistency of the thyroid was soft and elastic; it was moderately diffusely enlarged, the right lobe, however being somewhat larger N finger or tongue tremor The status at admittance, moreover showed signs of tonsillitis Body length, 101 cm, approximately 5 cm above the value for the age. Body weight 14 kg

LABORATORY FINDINGS

Serum cholesterol 175 mg/100 ml Protein-bound iodine concentration in serum before the beginning of the treatment 28 μg/100 ml. The concentration of hemoglobine 13 7 g/100 ml, leucocytes 20,000 of which polymorphonuclears 18,000. Non-protein nitrogen 57 mg 100 ml, the amounts of urine initially small and concentrated Urine sediment normal. Sodium, potassium and albumin in serum lay around the upper limit for normal values Standard bicarbonate 12 mEq/L Acetone 3+ Cerebrospinal fluid normal. N antibodies against thyroid hormone or thyroglobulin were found in the serum. She had normal amount of thyrotropin (TSH), less than 8 ME per 100 ml according to McKenzie method (11) On the other hand, four months after the crisis, very strong activity of "long-acting thyroid stimulator (LATS) was observed X-ray showed cranium, heart, and lungs normal. Skeletal development was advanced 67 ossification centers compared with the normal 49 ± 5.

THERAPY AND PROGRESS

The clinical picture agreed with thyroid crisis, and the patient was treated ith Lugol' solution (iodine 5 g, potassium iodide 10 g, aqua purificata 85 g) 1.5 ml in 100 ml 5.5 % glucose intravenously for 10 minutes; thereafter 1.5 ml Lugol' solution per liter 5 5 % glucose intravenously total of 2 liters glucose during the first 24 hours. Sodium lactate was added to counteract metabolic acidosis. Sulphonamides ere also administered and penicillin in large doses to treat the tonsillitis, as

LATS was estimated in the same way as plasma TSH but the response was measured 10 and 24 hours instead of 2 hours following the injection of plasma. LATS-activity was expressed semiquantitatively (15).

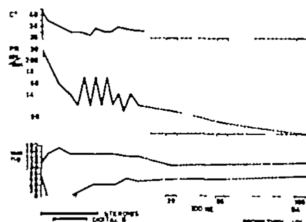


Fig 1 Temperature, pulse and blood pressure during the crisis days and during the following 3 months of treatment.

well as hydrocortisone intramuscularly 100 mg during the first 24 hours and thereafter in reducing dose. The hyperpyrexia was initially particularly resistant despite intensive antipyretic treatment with frequent acetylsalicylic acid enemas and washings with iced water The tachycardia was also persistent around 180 beats per minute for the first 24 hours of treatment, and the ECG indicated left loading and therefore the girl was given digitalis. Already after 2 or 3 hours of treatment, she brightened up and began to react when spoken to. In connexion with waking up, considerable motor restlessness and tremor set in, which was treated with phenobarbital sodium as sedative. During this treatment, general condition rapidly improved. Already during rst day of treatment she took liquids per os, on the day she could sit up in bed and feed herself, and on the third day she could look at story books. On the second and third day she had one liter of glucose, containing 3 ml Lugol' solution per liter per 24 hours intravenously On the fourth day the crisis was considered passed. Per oral iodine administration (Lugol / ml 4) and nutrition was begun. Propylthiouracil 50 mg per 10 kg body eight/day was introduced at the same time. The iodine was gradually discontinued during the following month. The corticoids were discontinued after one week, and digitalis after three days. Fig. 1 shows the fall in temperature in pulse, and in blood pressure after three days of treatment. These were normalized after three weeks, as was the patient' general condition in other respects.

Resin-triiodothyronine-¹³¹I-uptake according to Hvid Hansen's method (6) was, after 55 days of therapy 98 % (normal limits 82–115 %). The patient today after one year of treatment with propylthiouracil in maintenance dose of 25 mg × 2, is still euthyroid with Resin-T3-¹³¹-I uptake of 82% and PBI 7 1 μg/100 ml. Her goiter has increased slightly in size during the treatment period. Her eyes are no longer so protuberant. Her skin is dry and cool. She is now calm and can sit and concentrate on her play



Fig. 5 days after the onset of the thyroid crisis.

DISCUSSION

During recent years, several theories concerning the origin mechanism underlying thyroid crisis have been advanced in the literature.

Of these can be mentioned (*a*) sympatho-adrenal hyperactivity where the crisis is said to be caused by an acute adrenergic outbreak in the thyrotoxic patient, who is especially sensitive to sympatho-adrenal stimulation (3 19); (*b*) abnormally increased secretion of thyroid hormone (2, 14 13); (*c*) complete uncoupling of oxidative phosphorylation, i.e. an uncontrolled heatproduction is caused by the thyroid hormone blocking the adenosine triphosphate formation totally (5 8).

The most common causes of thyrotoxic crisis are nowadays infection, trauma, and mental strain in patients with thyrotoxicosis. In the case described above, it is without doubt the girl's tonsillitis that caused the thyrotoxicosis to erupt into a crisis.



Fig. 3 One year after the thyroid crisis.

Wiener & Lindeboom (22) distinguish two forms of thyrotoxic crisis: one a benign form with high PBI-values, the other a malignant form with normal PBI-values. The first is said to be a very severe thyrotoxicosis, which responds well to the ordinary thyrotoxicosis therapy: the other is said to be a peripheral form of thyroid crisis, which, as far as treatment is concerned, is much more difficult to manage. The present case, with its highly increased PBI-value of 28 $\mu g/100$ ml and its good restitution after treatment, can be considered to belong to the first type of thyroid crisis.

The effect of iodine therapy in thyrotoxic crisis is doubtful, but in this case a good restitution was obtained by treatment with Lugol's solution, low adult dose. The 10-year old girl with apathetic thyroid crisis, treated by Grossman & Waldstein (2), was not given any iodine therapy but only thiouracil. Their successful treatment results should support the supposition that it is not abnormally large amounts of thyroid hormone which provoke the crisis, and that the blocking effect of the iodine on the thyroxin secretion is not of decisive importance for the treatment of the crisis condition as such (17).

Propylthiouracil 50 mg per 10 kg body weight/day in tablet form was given on the fourth day of treatment, but is generally thought to be given as soon as the child can take medicine per os.

Corticoids were used in the present case but their effect could not be appraised. The opinions whether or not to use hydrocortisone for crisis treatment diverge. For most of the crisis cases (both adult and child material) described in recent years, steroid therapy has been employed. Those who advocate corticoids (1, 2, 1_, _0) consider that the good treatment result, with a downward trend of mortality in thyroid crisis from 70% on solely iodine therapy to 20–40 on the combination of iodine and corticoids, is due to an increased steroid metabolism in thyrotoxicosis, which during the crisis would produce a suprarenal cortex insufficiency. This would justify the administration of hydrocortisone. However others (7, 17), hold the opposite view based on the fact that in their cases of thyroid crisis, no signs of suprarenal cortex insufficiency have been found.

At the life-threatening condition posed by thyrotoxic crisis, the general opinion is that corticoids still belong to the therapeutic arsenal for the treatment of thyroid crisis.

Hyperpyrexia, which is an expression for uncontrolled heat production, must as early as possible be brought under control with the aid of antipyretics and frequent washings with iced water or diluted alcohol. Antibiotic treatment at the same time for possible infection is naturally important.

To control tremor and motor restlessness, phenobarbital sodium was in this case used initially. Later promethazine chloride was used with good therapeutic effect. We tried, with less effect, to treat the tachycardia and the threatening cardiac insufficiency with digitalis. The neonatal crisis cases, described in the literature (10, 13, 16), with cardiac insufficiency reacted well however to the administration of digitalis. Good result have been achieved in recent years with reserpine 0.1 mg per kg for children, or guanethidin 0.5 mg–2 mg per kg during 1–3 weeks, with the object of reducing sympatho-adrenergic

symptoms, such as tachycardia, hypertension, and tremor during the acute stage (21)

Fluid and electrolyte balance disturbances must, of course, be corrected, and a caloric and vitamin-rich nutrient administration, as well as oxygen, is essential The oxygen is given because of the considerably increased metabolism resulting in an increased need for it.

SUMMARY

A thyroid crisis in a 3 year old girl with previously untreated thyrotoxicosis and the treatment of it are described.

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CASE REPORT

NONSPHEROCYTIC HAEMOLYTIC ANAEMIA AND SEVERE JAUNDICE I A NEWBORN WITH PARTIAL PYRUVATE KINASE DEFICIENCY

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Hereditary nonspherocytic haemolytic anaemias not associated with the thalassemia syndromes or with hemoglobinopathy are usually connected with a defect in the activity of one of the enzymes upon which the erythrocyte depends for the normal function of its glucose metabolism. Deficiency in one of these enzymes, by cutting down the limited energy production of the red cell can result in red cell destruction and haemolytic anaemia.

More than 60 cases of hereditary haemolytic anaemia due to pyruvate kinase deficiency have so far been described (4 6, 7 8, 9 11 13 14 19 20 32, 35 36, 38, 39 41 47 48)

In at least 15 of these cases the disease has begun t birth with severe jaundice (6, 7 9 13 35 38, 39 47). The mode of transmission of this defect is autosomal according to Tanaka *et al.* (47), and only individuals homozygous for the defect should be liable to suffer from increased haemolysis, the heterozygotes presenting no clinical manifestation.

This paper reports a case of non-spherocytic haemolytic anaemia with pyruvate kinase defi ciency in the erythrocytes of a child who is probably heterozygous for this defect.

CASE HISTORY

Stefano E. is an Italian child born Oct. 1., 1966, and is now seven months old. He as first admitted to the pediatric clinic of the Ferrara University on the first day of lif because of severe asphyxia with diffuse cyanosis.

He had been born at term after an uneventful preg nancy the birth weight was 3500 g, blood group A Rh – Physical findings at birth consisted of cyanosis, hepatic enlargement, cm below the right costal margin, and splenic enlargement 1 cm below the left costal margin. Respiratory distress was also present, but after tw days of O_2 administration and medical therapy these symptoms completely receded

At the third day of lif rapidly increasing jaundice as noted; indirect serum bilirubin level was 28 mg 100 ml, direct Coombs test as negative The haematological data were Hb 10 g/100 ml, RBC 3,500,000, WBC 1.,400 with 60% neutrophils, 38% lymphocytes, 1% eosinophils, 1% monocytes. The platelet count was 150,000. Reticulocytosis of 9% Red cell morphology presented mild aniso- and poikilocytosis.

Red cell enzyme assay revealed that pyruvate kinase activity as about the normal value; other erythrocyte enzymatic activities ere slightly elevated, in agreement with the increased number of circulating reticulocytes.

An exchange transfusion with 400 ml of A Rh+ blood was performed. After the transfusion the serum bilirubin level dropped to 14 mg 100 ml. Gradually the icterus subsided At eleven days of age the patient was transfused ith 40 ml of A Rh – blood because of mild anaemia. The infant as discharged in good condition at 18 days of age

He has been examined weekly and has been referred on various occasions to the pediatric clinic for further investigations Physical findings at 7 months of age consisted of pallor and hepatic and splenic enlargement, (2.5 cm and 1.5 cm respectively below the costal mar gins). No skin or scleral jaundice was present. Body development had always been satisfactory The Hb was 7.6 g 100 ml, the RBC 4,000,000 the reticulocytes 17%. The hite cell count was 9600 with 20% neutrophils 76% lymphocytes, % eosinophils, and % monocytes. Enzymatic activities are reported in Table 3.

Abbreviations. PK pyruvate kinase, 3 DPG = 2 3 diphosphoglycerate.

Table 1 *Summary of haematologic data*

		Hb (g/ 100 ml)	Red cell counts	MCV (μ^3)	MCD (μ)	MCH ($\gamma\gamma$)	MCC %	Reticulocytes, ‰	Haematocrit, %	Osmotic fragility	HbA_2 %	Bilirubin D	Bilirubin I
Propositus	3rd day	10	3,500,000	92	—	28 3	31	90	33	—	—	—	21
	7th month	7 6	4,000,000	78	6.8	25	32	12	30	Normal	1 9	0.1	1.1
Mother		13	4,400,000	89	—	28.5	33	7	39	Normal	4	0.23	0 1
Father		10	3,860 000	84	—	26	29	10	32	Normal	1.8	0.15	0.2

Table 2. *Results of autohemolysis studies in members of kindred E*

% Hemolysis, 37 C, 48hrs

	Compounds added: None	Glucose (final concentration 0.75 %)
Propositus	2.7	3.7
Mother	2.23	1 7
Father	2.48	7 15
Normal range	0.4 − 3.5	0.5 ÷ 2

FAMILY HISTORY

The father (age 25) originates from Naples. He suffers from mild anaemia, asthenia and fatigue. These symptoms are not severe enough to impair his working capacity H has never been transfused.

Unfortunately it has been impossible to study the father's ascendants because his father died in a road accident, brother died at six months of age of sepsis, and the mother has not been available for study

The mother (age 19) originates from Ferrara. She has always been in excellent health. Her blood group is A Rh − During all the course of this pregnancy the indirect Coombs test was repeatedly tested and results were always negative.

We were able to study her mother and tw siblings. *A sibling* of the propositus, at seven days of age, was admitted to another pediatric hospital because of severe icterus. Direct Coombs test was negative, an exchange transfusion was performed and the icterus gradually subsided; he died at 27 days of age of severe, acute enteritis. N other haematological data has been available to us.

METHODS

Routine haematological studies were performed by standard laboratory methods. Autohaemolysis was performed

Table 3 *Enzyme activities in the red cell*

Values are expressed as micromoles of substrat converted per minute per g of hemoglobin

	Normal	Range	Propositus	Mother	Father	Uncle	Aunt	Grand-mother
Hexokinase	0.4	0.31–0 52	0.72	0.38	0.39	—	—	—
Phosphofructokinase	4 2	3.2–5 1	7	—	—	—	—	—
Fructose diphosphate-aldolase	2.2	1.8–2.6	2 3	—	—	—	—	—
Triosephosphate isomerase	345	325–400	410	—	—	—	—	—
Glyceraldehyde-3-phosphate-dehydrogenase	65	49–72	90	—	—	—	—	—
Phosphoglyceromutase	8 7	7–12	19 5	—	—	—	—	—
Pyruvate kinase	3	2.41–3.61	1	3 6	1 33	3.6	3.2	2.7
Lactate dehydrogenase	48	44–53	91	—	—	—	—	—
Glucose-6-phosphate-dehydrogenase	6.1	5 7–7 4	6.5	6.2	6.7	5.2	6	4.3
6-phosphogluconate-dehydrogenase	3	2.6–3 6	3 6	3	3.4	3 6	3.2	2.8
Malate dehydrogenase	48.5	48–50	82	—	—	—	—	—
Adenosine triphosphatase	4	3.6–4 7	6.1	—	—	—	—	—
Glutathione reductase TPNH dependent	2 08	1 7–2.4	2 3	—	—	—	—	—
Glutathione reductase DPNH dependent	0.66	0.63–0.78	1 02	—	—	—	—	—

according to Selwyn & Dacie (46) Haemolysates were prepared according to Busch *et al.* (14). PK was assayed according to Bücher & Pfleiderer with the modifications of *Brunetti et al.* (11), aldolase according to Bruns & Bergmeyer (12). Hexokinase, phosphofructokinase and adenosine triphosphatase were assayed according to Valentine *et al.* (49), glucose-6-phosphate and 6-phosphogluconate dehydrogenase according to Kornberg & Horecker (27), lactate and malate dehydrogenase according to Bergmeyer & Bernt (2, 3). Triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase and phosphoglyceromutase, were determined according to Kostrus *et al.* (28). Glutathione reductase as assayed with the modification of the method of Racker proposed by Beutler & Yeh (5).

GSH concentration was studied with the method of Patterson & Lazarow (40). ATP ADP and AMP concentrations ere determined using Boehringer kit; 2 3 diphosphoglycerate was determined according to Krimsky (29).

Haemoglobin starch gel electrophoresis was performed as described by Huisman (25). Quantitation of the various haemoglobin fractions was obtained by scanning the gel after staining with Amido Black in Joyce-Loebl Chromoscan and by elution of the unstained haemoglobin bands according to Aksoy & Harden (1). Cellulose acetate membrane electrophoresis of haemoglobins was performed according to Briere *et al.* (10)· the cellulose acetate membrane strips, stained with Ponceau S., were quantitated in the Chromoscan and duplicate sets eluted according to Rossman *et al.* (42).

All enzyme activities were assayed spectrophotometrically in Zeiss PMQ II or in Beckman DUB linked to Gilford Multiple Sample Absorbance System All assays ere performed at room temperature.

RESULTS

The clinical investigations and the researches performed on the propositus and on his parents gave the following results.

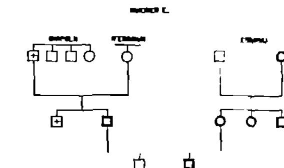


Fig 1 Pedigree ◪ heterozygous; + dead. Thick lines indicate subjects studied.

The blood smear and the osmotic fragility tested on various occasions, were always normal. Autohaemolysis test was not elevated, but the glucose addition resulted in an increased osmotic fragility and a more rapid rate of autohaemolysis (Table 2) Red cell glutathione was in the normal range (Table 4) and no abnormal haemoglobin was detected with starch gel electrophoresis. No pathological modification in the activities of the red cell enzymes tested was detected (Table 3) except for PK, whose activity was 30% of the mean range and phosphoglyceromutase which presented a marked increase. The blood ATP and the ATP/ADP ratio, as well as the ATP consumption after 24 hours incubation at 37 C were in the normal range, as observed at 5 and 7 months of age; the level of 2–3 DPG presented a conspicuous increase (Table 4).

The family studies revealed that a similar meta-

Table 4 *Concentration of red cell compounds*

Values are expressed as mg/100 ml whole blood corrected for 40% haematocrit except for 2-3-diphosphoglycerate hich is expressed as mμ moles per ml of packed fresh red cells

	Mean	Range	Propositus	Mother	Father
GSH	30	18–40	22	27	40
2 3, DPG	2500	2000–3000	5500	2600	4540
ATP	25	10–28	23	23	21
ADP	4	2–6	4.23	3 68	5.52
AMP	1	0.5–1 5	1 2	1 65	1.05
After incubation at 37 C for 24 hrs					
ATP	7	4 5–8	7 3	4.5	6.3
ADP	2.5	1 3	2.2	1 84	1
AMP	0.2	0 05–0 8	1	0 07	0.075

bolic defect (PK activity 43% of the mean, normal autohaemolysis increased by glucose addition and increased 2–3 DPG) was present in the father's erythrocytes, while the mother had a normal PK activity autohaemolysis test and 2–3 DPG concentration. PK activity was normal also in all the mother's living relatives (Table 3).

DISCUSSION

The propositus at the third day of life presented a severe icterus which required an exchange transfusion. After this event the patient presented only a slight nonspherocytic anaemia which, up to the present time, did not require any clinical treatment.

Blood group incompatibility hereditary spherocytosis, abnormal haemoglobins (24) low level of glutathione (37) the presence of deficiencies of certain enzymes which can be responsible for haemolytic anaemias as, hexokinase (49) triose phosphate isomerase (43) diphosphoglyceromutase (7 31 34 44), glucose-6-phosphate dehydrogenase (16, 17) 6-phosphogluconate dehydrogenase (30 45 50), adenosine triphosphatase (23) and glutathione reductase (15 33) can be excluded in the pathogenesis of the haemolytic anaemia presented by the propositus.

On the evidence of the data obtained studying PK activity in the patient and in his parents, [illegible]an be suggested that the patient is a hetero-[illegible] for the PK defect. In fact in our case the conditions which Tanaka *et al.* (47) consider typical of the heterozygote are present, i.e. enzymatic activity reduced to about half the normal range and presence of the defect in only one of the parents. Beside, the high level of 2–3 DPG observed in our case is typical of the PK deficiency and in accordance with this interpretation.

The normal level of red cell ATP which our case presented is not common in carriers of PK deficiency but has already been described in other cases (26 38)

By the autohaemolysis test, our case could be classified as type II of Selwyn & Dacie, according to the observation of Grimes *et al* (20).

On this evidence two hypothesis can be suggested to explain the pathogenesis of the icterus observed. The pyruvate kinase defect can be considered casually present in the propositus and the icterus attributed to another metabolic defect which we were not able to detect. The second possibility is that this deficit, although partial, caused the severe haemolytic crisis found at birth.

The data of Tanaka *et al* (47), who did not find clinical manifestations in the heterozygotes observed, are against this hypothesis.

Nevertheless from a review of the literature pertinent to hereditary haemolytic anaemias due to pyruvate kinase deficiency we were able to find at least two cases where probably the proposti are heterozygotes for the gene (4 14 21 22, 38) In fact Bestetti *et al* (4) observed a slightly severe anaemia in a subject with a defect in the red cell PK, whose father was heterozygous for the defect, while the mother's PK activity was at the lower limit of the normal range.

More recently Busch *et al* (14) described a case of severe haemolytic anaemia with icterus which required an exchange transfusion in a newborn with a partial PK defect and a similar genetic background. Two other cases of slight nonspherocytic haemolytic anaemia with mild PK deficiency have been described by Oski & Diamond (38) and by Gross (21 22).

Our case differs from these cases because the severity observed at birth was only transitory at the present time our patient presents a moderate anaemia which does not require medical therapy or transfusion.

We should also like to point out that the case presented by Busch *et al.* (14), was probably also a carrier of a partial glucose-6-phosphate dehydrogenase defect, and this association could be responsible for the more severe clinical pattern observed by these authors.

These observations and the data of the literature give support to the hypothesis that a partial defect of red cell PK can also, in certain cases, be responsible for clinically evident haemolysis. This could more easily arise in particular conditions (as the neonatal period) where normally an increased haemolysis is present.

SUMMARY

We present a case of familial non spherocytic haemolytic anaemi which began with severe neonatal jaundice (I.B. 28 mg/100 ml). The propositus presented a partial PK deficiency which was also present in the father but not in the mother

We were able to exclude other conditions which can be responsible for haemolytic anaemia, and neonatal jaundice.

The hypothesis that also a partial PK deficiency can, in particular conditions, be responsible for a severe haemolytic process, is suggested.

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Acta Pædiat Scand 57 65–71 1968

PROCEEDINGS OF PEDIATRIC SOCIETIES

DANISH PEDIATRIC SOCIETY

Meeting Oct. 12, 1966

Karen Ryelund & Bent Nielsen *Acute glomerulonephritis in an infant aged four months.* (To be published in *Acta Pædiat Scand*)

A case of acute oliguric glomerulonephritis in an infant aged four months was described. The presenting symptoms were afebrile seizures after an illness of approximately ten days with uncharacteristic symptoms.

Initially because acute encephalitis was suspected, the patient was briefly treated with corticosteroids. When the diagnosis of acute glomerulonephritis was established, the patient was treated with restricted fluid administration and peritoneal dialysis. During dialysis, the condition improved considerably and the seizures ceased. A few days later the diuresis occurred and the uremia disappeared. The infant could be discharged six weeks later with minimal proteinuria, serum creatinine of 0.5 mg/100 ml and a systolic blood pressure of 90 mm.

The treatment administered was discussed and it was concluded that, in acute oliguric glomerulonephritis, there are good indications for dialysis whereas the indications for steroid treatment are not yet firmly established.

Discussion

The differential diagnosis was discussed. *B Friis-Hansen* inquired whether pyelonephritis might have been present. *N Hoboth* drew attention to the possibility of hereditary nephropathy and *H T Lund* mentioned the congenital nephrotic syndrome. *Bent Nielsen* replied that the biopsy did not reveal inflammatory changes. A hemolytic uremic syndrome had also been considered. *P W Bastrup* emphasized that the findings did not entirely suggest an acute condition.

Anne Nygaard & Tyge Cl. Gertz: *Atresia of the oesophagus and neonatal ileus*

From 1960 till 1966, seven cases of atresia of the oesophagus were operated upon. In all of the cases the most common form was present with the upper segment ending blindly and a fistular opening between the lower segment and the trachea just over the bifurcation. In four of the cases hydramnios had been present in the mother. All of the infants were submitted to operation within the first day of life. Thoracotomy on the right side was undertaken. The fistula was closed with a ligature after which end-to-end anastomosis was undertaken with a single row of silk ligatures. The thorax was closed without drainage. Two infants died immediately post-operatively (congenital heart disease and aortic stenosis caused by the suturing). The remaining five infants have developed normally and only one of them has had slight symptoms caused by slight oesophagitis.

Neonatal intestinal obstruction is a relatively uncommon condition. Between 1960 and 1965 we operated upon a total of 20 cases. The cases consisted of six cases with intestinal atresia (duodenal atresia in 3, jejunal atresia in 2 and atresia of the ileum in 1), two infants with malrotation, four infants with volvulus and eight infants with meconium ileus of whom three had mucoviscidosis. In the group with atresia, the lethality was 0 and the children have developed normally with the exception of one child in whom congenital cardiac disease is also present. One of the infants with volvulus died, the entire intestine being gangrenous. The three infants with mucoviscidosis died and the remaining five children have developed normally.

In the entire material, the lethality was low

and we attribute this to the rapid diagnosis and the possibilities for early operation.

Discussion

E. Thamdrup inquired whether any of the patients with mucoviscidosis had received treatment with pancreatic enzyme. *A Nygaard* replied that these infants died before such treatment could be instituted.

Henning Andersen drew attention to the "H-type with isolated tracheo-oesophageal fistula.

J Vesterdal recommended a follow-up investigation of the extensive material of oesophageal atresia from Rigshospitalet.

Torben Iversen emphasized the great incidence and the diagnostic significance of hydramnios in atresia of the oesophagus.

N Hobolth, G Buchmann & L. E. Sandberg: *Congenital choanal atresia.* (Published in *Acta Pædiat Scand 56* 286, 1967)

Discussion

J Vesterdal The fact that choanal atresia not only involves a risk at delivery but also later on [illegible] from the following case history A female [illegible] with a birth weight of [illegible]240 g presented difficulties in respiration and cyanosis from [illegible] and choanal atresia was demonstrated when the child was one day old. The respiratory difficulties were partly due to the fact that the child sucked the tongue down into the throat. When the infant was three months old, the posterior wall of the left choana was perforated operatively with a drill but it did not prove possible to keep the opening patent despite introduction of a plastic drain. Three weeks later the opening was enlarged at re-operation. This resulted, however in aspiration of secretion from the left nasal cavity into the respiratory tract and the child developed repeated episodes of aspiration pneumonia and died from such an infection one month after the last operation.

Thus, I must agree with Dr Hobolth that it is best to postpone operation until the child is a great deal bigger

P W Brestrup The incidence of choanal atresia encountered is greater than anticipated. Probably a number of cases are overlooked. The passage of a nasal catheter is of value. Otherwise, the clinical picture is quite typical.

S. Sparrevohn *Familial eosinophilic collagenosis.* (Published in *Acta Pædiat Scand 56* 307 1967)

Discussion

K Wilken-Jensen considered that the diagnosis of food allergy could scarcely be excluded and that a milk free diet for only a week was of no diagnostic value.

After the meeting the ordinary general meeting was held. The following were re-elected. Henning Andersen (chairman) B. Friis-Hansen (deputy chairman) and Torben Iversen (secretary).

Meeting Nov 11 1966

J Helweg-Larsen. *A case of visual agnosia*

E. B. Buch: *Idiopathic infantile hypercalcemia*

A case of idiopathic infantile hypercalcemia in a boy aged 2 1/2 years was presented. He had shown hypothrepsia, vomiting and constipation from the age of 6 months. At the age of 11 months, hypoplasia of the right and left branches of the pulmonary artery associated with increased pressure in the right ventricle were demonstrated. Since then, the child has been repeatedly admitted to this department and, on each occasion, hypercalcemia varying between 12.1 and 14 1 mg/100 ml has been demonstrated and the patient has shown psychomotor retardation and hypotonia but without actual cardiac symptoms. During the most recent admission, his striking elf-like appearance was noticed and the diagnosis was then established. The patient was then treated with a low-calcium diet without supplementary vitamin D but this did not result in clinical improvement or fall in serum calcium. The treatment was, thereafter supplemented with cortisone and this resulted in normalisation of the serum calcium in the course of six weeks. During this treatment, the blood urea, similarly returned to normal.

Discussion

J Flamand Christensen referred to a similar patient with serum calcium of up to 14.5 mg/100 ml, who was treated with prednisolone.

During the discussion the question of the mode of action of cortisone in this condition was raised (increased calcium loss in the urine, delayed resorption of calcium from the intestine) but no satisfactory explanation was reached.

Erling Nathan: *Coronary occlusion in childhood*

Coronary occlusion resulting from atheroma was the cause of death in two children treated in Rigshospitalet, Copenhagen.

A boy aged 13 years had suffered from epilepsy since the age of 8 years and was treated for hypophyseal insufficiency. Terminally he had severe cardiac insufficiency. The lactic acid dehydrogenase was raised. The blood pressure was normal. Neither pain nor typical ECG changes were present. Autopsy revealed myocardial fibrosis and recent infarction resulting from atheromatosis of the aorta with stenosis of the coronary ostia. In addition, there was also congenital, partly degenerative, possibly viral, cerebral damage.

A girl aged 5 $^{3}/_{4}$ years had raised blood pressure caused by pheochromocytoma. She experienced attacks of precordial pain with dyspnoea and paleness. In addition she had anorexia and polydipsia/polyuria. Terminally severe heart failure was observed with raised lactic acid dehydrogenase and an infarct pattern in the EEG. Autopsy revealed thrombosis in atheromatous coronary vessels and pheochromocytoma.

Coronary occlusion in children is seen with abnormal origin of the coronary vessels, in congenital necrosis of the media, in disturbances of calcium metabolism, in Friedreich's ataxia and Refsum's disease with subintimal fibrosis, in rheumatic fever and in periarteritis nodosa. Atheromatosis may be observed in children with raised blood pressure, familial hyperlipoproteinemia, myxoedema, diabetes mellitus and progeria. The dietary fat-content, adrenaline production and infections, similarly are considered to be of significance. In pheochromocytoma in children, in addition to attacks of headache and precordial pain, the following symptoms may be observed: polydipsia/polyuria, anorexia, nervous symptoms and signs of genito-adrenal dysfunction. The blood pressure becomes stationary at a raised level. The serum protein bound iodine is frequently high.

Discussion

J Flamand Christensen mentioned a child aged two years with severe heart failure, hypertension, severe ECG changes and pronounced atheromatosis at the beginning of the coronary arteries.

Henning Andersen commented upon the hypophyseal insufficiency in the patient mentioned first above.

Inge Tygstrup, H. E. Christensen, Børge Sørensen & B. Zachau-Christiansen. *A case of listeriosis in a newly born infant*

A pregnant woman aged 25 years developed an influenza-like, apparently endemic, infection 2-months before the expected delivery. After a symptom-free interval of 3 weeks, she again developed slight symptoms together with slight pyuria, went into labour and after pains lasting for a few hours she gave birth to a boy weighing 2150 g with asphyxia. During the four hours which he survived he did not cry properly and practically constant artificial respiration was required. During this, he developed generalized hemorrhages into the skin, was anemic with agranulocytosis and slight thrombocytopenia. Autopsy and microscopic examination suggested that the infant had a congenital *Listeria* sepsis. It was discussed whether the agranulocytosis was (1) Kostmann's hereditary form (2) was due to leukocyte agglutinins demonstrated in the maternal blood (3) was secondary to sulphonamide treatment in the mother or (4) due to the bacterial infection.

Discussion

J Vesterdal mentioned a case observed in the pediatric department, Rigshospitalet, Copenhagen, in about 1960.

H Ekelund (Malmö) referred to a material of Listeria infections in Uppsala in 1959–1961 in foetuses and newly-born infants (*Acta Paediat Scand 51* 698, 1962) and emphasized the periodic occurrence of infection in the published and other Swedish cases. Since then no further cases have been discovered despite intensive investigations.

B. Zachau-Christiansen *Salicylic acid poisoning in th ee infants*

Three serious cases of salicylic acid poisoning in infants endangering life were reported. Two of the patients survived without sequelæ while in one patient blindness and severe brain damage developed. This patient received 5–10 g in 36 hours, the second patient half of this dose and the third 4 1/2 g in 72 hours.

Probably even severe cases can be treated without dialysis but with intravenous infusions of bicarbonate alone which was administered in the third case. Administration of potassium is also essential. It is recommended that treatment be undertaken where the dialysis department and the pediatric departments can work together as many physicians would not dare omit dialysis which was employed in the two other cases reported here.

The wisdom of treatment of common infections and painful states in infants and small children with salicylic acid is discussed. Such treatment is unnecessary in principle but is established.

The dosage of acetylsalicylic acid up to the age of five years is 50–75 mg per year of life, not oftener than four times per 24 hours and not for longer than three days.

Confusion between junior aspirin of 150 mg adult aspirin of 500 mg is warned against. ...usion such as this was the cause of poisoning in two of the cases while in the third case the child had received soluble acetylsalicylic acid tablets.

Discussion

H. C. Berthelsen (Dialysis Department, Rigshospitalet) emphasized that the lethal concentration of salicylic acid is 50–60 mg/100 ml and that this is an indication for emergency hemodialysis.

E. Thamdrup considered that junior aspirin should be withdrawn because the public can easily get the impression that these are without danger.

J. Helweg-Larsen & E. Jacobsen. *Treatment of spasticity with peripheral nerve blockage*

K. Mauritzen & H. Borch Nielsen. *Colostomies in children, review of a 15 year material*

Very divergent opinions exist concerning the incidence of complications of and the mortality from colostomy in childhood. In order to illustrate this, a material of 92 cases of colostomy in childhood is analysed.

Emergency colostomy was undertaken for neonatal intestinal obstruction, on account of Hirschsprung's disease or colonic or anal atresia.

In cases of Hirschsprung's disease where conservative treatment with rectal enemata was unsuccessful colostomy was undertaken at a later date. In the remaining cases colostomy was performed a couple of weeks prior to radical operation at the age of 1–2 years.

In cases of atresia of the anus, neonatal colostomy is most frequently indicated in boys as girls with recto-vaginal fistulæ have satisfactory passage following dilation.

Colostomy is as a rule placed corresponding to the right side of the transverse colon or more rarely corresponding to the sigmoid colon or in occasional cases, corresponding to the cæcum on account of intestinal perforation.

The average duration of colostomies for atresia was 16 months and for Hirschsprung's disease 9 months.

Complications occurred in 51 patients. A fourth of these consisted of prolapse of the afferent intestinal limb which did not cause symptoms except in one case of mechanical obstruction.

Retraction occurred in 12 cases and 8 of these were treated with glass rods which were removed after a week. In cases where the glass rod was in situ for more than three weeks, there was no retraction.

Irritation of the skin was encountered surprisingly rarely. Abscesses occurred in five cases in two of which the intestine was not fixed to the abdominal wall.

Opening of the intestine was undertaken primarily when the colostomy was formed in half of the cases without increased incidence of abscess formation (3 and 2 cases, respectively).

None of the patients died as the result of the colostomy.

Fixation of the intestine to the abdominal wall (peritoneum, fascia or skin) was carried out in 14 out of 25 cases with prolapse of the intestine and did not prevent this occurring.

Closure of the colostomy resulted in fewer but more serious complications. Five patients developed ileus which was of mechanical origin in three cases and in one case insufficiency of the

anastomosis proved fatal. The remaining colostomies were closed without complications but the blood loss was not inconsiderable, frequently between 100 and 200 ml, and greater the longer the colostomy had been present.

Colostomy in childhood causes a number of insignificant complications when it is performed while the complications on closure are fewer but more serious. Delay in performing colostomy is unjustified as the risks involved in postponement are considered to be much greater

A number of children die after colostomy not because of but despite this. In the present material, nine children died. The causes of death were reviewed.

Discussion

C M Madsen recommended that in Hirschsprung's disease, the colostomy be placed as distally as possible on the part of the intestine which is normal as regards the presence of ganglion cells. In the majority of cases, this is so in the sigmoid colon. The colostomy may then be resected at the actual operation and a third intervention, viz. closure of the colostomy is avoided. In addition, Dr Madsen recommended Nixon's method of colostomy in newly-born infants and described the technique.

K Mauritzen. I am familiar with Nixon work and I have seen him employ the technique in London and have discussed it with him prior to publication. The original indication was prevention of retraction. The use of a plastic rod for a sufficiently long time or permanently had the same effect. A bridge of skin does not prevent prolapse but perhaps it is less common. Prolapse only causes slight symptoms, however and colostomy with a skin bridge may be difficult to close.

As regards the localisation of the colostomy-Some surgeons prefer colostomy in the sigmoid colon.

(1) in order to avoid an extra operation, viz., closure of the colostomy as the sigmoid colon is drawn to the anastomosis in radical operation for Hirschsprung's disease or is closed during radical operation for atresia of the anus,
(2) the intestine proximal to the colos may develops better
(3) the children thrive better and
(4) diarrhoea is avoided.

As regards point (1) I consider that transversostomy reduces the risk of abscess formation around the anastomosis in Swenson's operation or closure of the sigmoidostomy in atresia of the anus and prevents serious complications if thi or stenosis should occur i.e. complications (peritonitis or ileus) which are indications for emergency transversostomy under favourable conditions.

Re (2), we have not had trouble with too short, underdeveloped sigmoid colons during radical operation for Hirschsprung's disease or operations for atresia of the anus despite transversostomy performed neonatally

Re (3) our children with neonatal transversostomies thrived normally

Re (4) diarrhoea after transversostomy occurred only rarely and was of short duration in our patients.

Meeting Dec. 16, 1966

Christmas meeting with ladies in Domus medica. Photographs and impressions from the congress in Tokyo by P W Brestrup P Plum, N J Schierbeck and J Vesterdal.

Meeting Jan. 27 1967

Discussion of the proposals for the new recommendations for specialist education.

Meeting Febr 10, 1967

J Vesterdal *Experience of prolonged visiting time in children department* (Published in *Ugeskr Læg 129* 931 1967)

Since the opening of the pediatric department in Glostrup in October 1965 the visiting hours have been daily from 2–7 p.m. which is a good deal longer than is usual in pediatric departments in Denmark. Parents have been very satisfied with this arrangement and it is our impression that it has improved the hospital environment for the children. The staff have had to learn to put up with the disadvantages associated with this arrangement.

Discussion

C Hansted stated that in the pediatric department in Aalborg Municipal Hospital, siblings are allowed to visit provided that they are healthy and have not been exposed to infections.

Henning Andersen did not consider that any particular risk was involved in this.

E. Thamdrup reported that in the pediatric department in The Central Hospital in Hillerød the visiting time was three hours daily and that this did not involve difficulties.

P Plum asked how long the average duration of a visit was in Glostrup. *J Vesterdal* could not give any precise information.

J Vesterdal *The battered-child syndrome*

The case histories of four infants were reported. These infants were admitted on repeated occasions with various symptoms (bruises, concussion, fractures, attacks") which could not be explained by any demonstrable disease in the infants. When this is compared with the form and the extent of the bruises etc. it is concluded that maltreatment must have occurred. It is emphasized that maltreatment of infants is of great danger to them and that it is therefore important that the condition is diagnosed. It is necessary to notify these to the Child Welfare Authorities and it is also recommended that the local Medical Officer of Health be notified.

Discussion

H Gormsen quoted a series of fatal cases of maltreated children from the Institute of Forensic Medicine in Copenhagen. It is important that doctors become aware of this syndrome which has probably always existed (subdural hematomata, multiple fractures etc.). It is important to undertake radiographic examination of the entire skeleton in suspected cases.

B Lindquist (Lund): In Sweden, it is difficult to accuse or punish the responsible individuals in such cases unless the child has died.

P Plum. It is important not to accuse the parents unjustly. Diseases such as the hemorrhagic diathesis, scurvy osteogenesis imperfecta must be excluded by all possible means if the doctor is to express any opinion in court on the subject.

K. Biering-Sørensen. The diagnosis may be very difficult in many cases. It should be noted whether hair has been pulled out.

Sv Heinild enlarged upon the expression, punishment of children, and said that it was his experience that 90 % of parents on detailed questioning stated that they had employed corporal punishment for their children "when they had deserved it

P A. Krasilnikoff· *Albumin metabolism in normal children and premature infants*

Albumin metabolism was investigated with human albumin labelled with ^{131}I in 23 normal children aged from eight days to 13 years, and in four premature infants. The results were compared with similar results in 27 adult patients investigated by N Rossing.

A lower albumin concentration was found in the serum but a distinctly higher plasma volume per kg body weight in the premature infants and normal infants as compared with the older children and adults. The intravascular albumin mass expressed per kg body weight was found to be constant throughout the entire period of childhood (approximately 2 g per kg).

The relative rate of catabolism (= the percentage of the intravascular albumin mass which is broken down daily) showed a distinct fall from approximately 19 % in premature infants to about 8 % in older children and adults. Correspondingly a greater rate of synthesis of albumin was found in premature infants (approximately *0.50* g/kg/24 hours) and normal infants (approximately 0.34 g/kg/24 hours) compared with older children (approximately 0.17 g/kg/24 hours) and adults (0 15 g/kg/24 hours).

Finally a significant linear correlation was demonstrated between synthesis (g/24 hours) and the body weight from birth and until adult life.

From this it is concluded that the low serum albumin concentration in the smallest infants must be considered to be a dilution phenomenon.

Discussion

B Friis-Hansen. The calculations are based upon a "steady state but infants are not in a "steady state at all as they are growing, at the rate of

1–2% per 24 hours and correction must be made for this.

H. Sardemann. *Spontaneous hypoglycemia treated with diazoxide*

The effect of diazoxide, a benzothiadiazide derivative with a hyperglycemic affect, was tried in prophylactic treatment of a tendency to hypoglycemia in idiopathic spontaneous hypoglycemia in a boy aged one year who had presented serious therapeutic problems. Frequent meals, supplementary glucose, leucine-poor diet, sodium glutaminate, glucagon and antiepileptic drugs had not produced any significant change in the frequency of the attacks or the fasting blood sugar. During treatment with 160 mg diazoxide daily supplemented with 1 mg trichlormethiazide (Fluitran) daily to increase the hyperglycemic effect, the incidence of attacks has diminished and the patient's fasting blood sugar appears to have adjusted itself to a higher level. During the three months in which this treatment has been administered the patient has developed hirsutism but there have been no other side-effects in the form of gastro-intestinal symptoms, involvement of the circulatory system, fluid or salt retention or raised serum uric acid.

Discussion

J. Melchior: If the blood sugar cannot be maintained at a suitable level, treatment with cortisone should probably be attempted.

T. Miletic & M. Egeblad. *Late perforation after radiographic reduction of intussusception*

Perforation in connection with reduction of intussusception of the small or large intestines under radiographic control is stated to be extremely rare. In the pediatric department, The County Hospital in Glostrup, one case of perforation of the colon was observed. The patient was a girl aged 5 months who had previously been healthy. Four days prior to admission, she was vaccinated with triple- and polio-vaccines after which she was restless and cried a great deal with pyrexia of 38°C for two days. For the 12 hours immediately prior to admission she was restless and crying. Two hours prior to admission there were brief attacks of screaming and a single normal bowel motion. Just before admission bloody stools were passed and twelve hours later the general condition deteriorated. At that time, an abdominal swelling could be felt and there was blood on the finger after rectal examination. Ileocolic intussusception was demonstrated radiographically and reduced by hydrostatic pressure without difficulty. Approximately 12 hours later the child again became restless with thin foul-smelling stools and generalized tonic and clonic seizures. During the subsequent three days the general condition was poor. The abdominal circumference increased. On the fifth day after reduction, radiograph of the abdomen revealed pneumoperitoneum. At laparotomy copious amounts of air were found in the peritoneum and a perforation demonstrated in the transverse colon which had probably occurred some time previously. The post-operative course was uneventful.

The reason for the perforation could not be demonstrated. Possibly the intussusception had occurred earlier than the history suggested. Another possibility was perforation in connection with renewed intussusception.

It is recommended that the history is taken meticulously in view of the age of the intussusception, before hydrostatic pressure reduction is attempted.

Torben Iversen

Acta Paediat Scand 57 72–86 1968

PROCEEDINGS OF PEDIATRIC SOCIETIES

THE EUROPEAN SOCIETY FOR PAEDIATRIC ENDOCRINOLOGY

Abstracts of the Papers Read at the Sixth Annual Meeting Haifa, Israel (Kupat Holim and Tel-Aviv University Medical School)

March 19th–24th, 1967

INTRODUCTION

The Sixth Meeting of the European Society for Paediatric Endocrinology was held at the Ben Dori (Meggido) Recreation Home of Kupat Holim. The main topics were:

Perinatal Endocrinology (Chairman. Dr A. Prader Zürich).

Thyroid disorders (Chairman Dr M Pierson, Nancy).

Sterolds (Chairman. Dr H. K. A. Visser Rotterdam).

Adipose tissue—obesity (Chairman. Dr D Hubble, Birmingham)

Pituitary (Chairman. Dr J R. Bierich, Hamburg).

[illegible] extracurricular session was held on "Child [illegible] in the Kibbutz.

The number of active participants was 57 coming from 16 countries. The number of associated members was 30

We wish to express our appreciation for the financial support received from Choay Ciba, Kupat-Holim, Nutritia, Organon and Schering.

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Claude A. Villee (Harvard Medical School intr by Zvi Laron) *The placenta and fetal tissues a cooperative enterprise for the synthesis of steroids*

A woman in the latter months of pregnancy synthesizes some 250 mg of progesterone and 30 mg of estrogens. Some problems of current interest are the nature and sequence of the biosynthetic reactions involved, the tissues in which these reactions occur and the means by which the several steps in biosynthesis are regulated. The problem has been studied using tissue slices, homogenates or cell-free preparations of the placenta, cotyledons or entire placentas perfused after removal from the body or while the placenta is still in place in the uterus, or by perfusion of the previable fetus after its removal at a therapeutic interruption of pregnancy

The placenta has all the enzymes to convert acetate to cholesterol and cholesterol to progesterone Mevalonic acid, prenol pyrophosphates, squalene, lanosterol and cholesterol are intermediates. In contrast, the placenta has little or no enzymatic activity for the conversion of progesterone or pregnenolone into androstenedione or dehydroepiandrosterone and no 16-hydroxylase. The two enzymes missing from the placenta are found in high concentration in the fetal liver and adrenal. The absence of the enzyme for converting C^{21} steroids to C^{19} steroids probably accounts for the failure of attempts to demonstrate the overall *de novo* synthesis of estrogens by the placenta. The placenta has a very active aromatizing enzyme system for converting androgens to estrogens.

Fetal tissues, especially the fetal adrenal have a very active enzyme for adding sulfates to steroids. In contrast, the placenta has little or no activity in sulfurylation but a very active sulfatase enzyme. These experiments are consistent with the hypothesis that steroids such as DHA are sulfurylated in the fetal adrenal, pass to the placenta as the sulfates, and then are desulfated i the placenta. The fetal liver can convert progesterone to estradiol and estradiol to estriol. Thus, the fetal liver and perhaps the fetal lung may be important components of the feto-placental unit.

Dorothy B. Villee (Harvard Medical School intr by Zvi Laron): *Control of steroid hormone synthesis in human f tal adrenals*

Human fetal adrenals have little or no 3 beta-hydroxysteroid dehydrogenase, but abundant 17 and 16alpha-hydroxylase, *in vitro* The enzymes involved in 21 and 11-hydroxylation appear to develop later in gestation, so that, with progesterone as substrate, the pattern of steroid synthesis differs with gestational age. Progesterone added to the medium is capable of altering the enzymic activity of human adrenals in organ culture in a manner suggestive of the phenomena of induction and repression in microorganisms. Recently human fetal adrenals have been maintained in organ culture with added mouse testicular RNA. The tissue, after 24 hours exposure to testicular RNA, showed enzymic activity compatible with the origin of the RNA, i.e. 3 beta hydroxysteroid dehydrogenase activity was readily demonstrable, and more androgen was formed when compared with adrenals cultured in nutrient medium alone. Experiments with radioactive RNA extracted from adrenals and testes of rats injected with tymidine-^{3}H give evidence that the RNA is taken up by adrenals in culture and can subsequently be extracted from the tissue as intact RNA. Thus, it is possible to alter the pattern of steroid synthesis in one gland in organ culture by the addition to the medium of biologically active RNA from another steroid hormone producing gland. (Supported by USPHS grant AM 08026.)

Rolf P Zurbrügg (University Children Hospital Berne; intr by A. Fanconi): *Steroidal rhythmicity in neonates and early life time periods*

Rhythmicity is a basic characteristic of life and demonstrates that all organisms are structures not only in space but also in time. The pectrum of physiologic rhythms in the human is broad and many different frequencies in the adult are known.

One of the most impressive circadian cycles is the fluctuation of plasma cortisol levels. As it is true for all biologic phenomena a maturing and aging developmental pattern is very likely to exist also for this adrenal cycle.

We have investigated the periodicity of plasma cortisol concentrations in various early life tim periods. Examining frequencies, amplitudes, th overall level around which cycling occurs, marked differences in comparison with the rhythmicity in the adult could be stablished. Similarities t physical oscillators as well as probable rhythm phasing, synchronizing and desynchronizing factors will be discussed.

Fabio Sereni, Lucia Piceni Sereni, Nicola Principi, Giuseppe Chiumello & Ottavio Branabei (Pediatric Clinic, University of Milano intr by Andrea Prader): *The rol of adrenal ortex ontrolling li er RNA sy thesis during the perinatal period of li*

Soon after birth a sharp increase of the rate of liver RNA synthesis occurs. Two series of data obtained in our laboratories support this statement. *I vo* when radioactive precursors (6-^{14}C orotate, ^{14}C-uridine, ^{32}P) are injected intraperitoneally to newborn rats or rabbits, the specific activity of rapidly labelled nuclear RNA increases manyfold few hours after birth. *In ro* the activity of DNA dependent RNA polymerase of isolated nuclei from rat liver also increases in a very significant degree after birth. Further studies investigated the role of adrenal cortex in controlling the rate of liver synthesis and metabolism in the perinatal period. Adrenalectomy performed soon after birth is effective preventing, at least in part, the sharp postnatal increase of the rate of 6-^{14}C-orotate incorporation into liver nuclear RNA. The administration of glucocorticoids to intact newborn rats does not influence the DNA dependent RNA polymerase activity of isolated uclei during the first 12 days of extrauterine life. However starting from the fourth day after birth the rate of incorporation of radioactivity from 6-^{14}C-orotate into liver uclear RNA is clearly increased by hydrocortisone.

It is suggested that soon after birth glucocor

ticoids play a role stabilizing nuclear RNA. Preliminary data on the influence of hydrocortisone on liver nuclear (and cytoplasmic) RNase activity seem to support this hypothesis. The possible significance of our data in relationship to the postnatal activation of liver protein synthesis and to the increased activity of a number of enzyme systems soon after birth will be briefly discussed

This work was supported by research grants from the National Institute of Health (HD-01895) and by the Association for the Aid to Crippled Children, New York.

P Malvaux, Ph. De Mayer C. Beckers & M De Visscher (Laboratoire de Pathologie Générale, Université de Louvain) *Free thyroxine in maternal and cord blood*

Considering the placenta only as a membrane permeable to the free thyroxine present in serum, it is possible to calculate the level of free thyroxine (T_4) in maternal and cord blood and the transfer of thyroxine from foetus to mother or vice versa.

The free thyroxine level and the caracteristics of the thyroxine binding proteins (TBG and TBPA) have been determined on blood samples taken from the mother and cord blood at the time of delivery

The values of T_4 are not different in the new as compared to the mothers while the maximal binding capacity of TBG and TBPA is significantly reduced in the newborns.

It is likely that at the end of pregnancy there is an equilibrium between maternal and fetal free thyroxine.

E. Gautier E. Juillard & T Lemarchand-Béraud (Clinique Infantile Universitaire et Département de Biochimie Clinique, Clinique Médicale Universitaire, Lausanne) *Neonatal thyrotoxicosis*

Case 1 The mother acquired Basedow's disease at the 6th month of pregnancy and was treated before delivery The infant was born at term. Thyrotoxicosis gradually developed reaching its peak at the 17th day of life. No treatment was needed and the symptoms abated by 6 weeks. The presence of LATS could be demonstrated in the serum of the mother and of the infant.

Case 2 The mother acquired Basedow's disease one and a half year before the birth of the infant. Partial thyroidectomy was performed one year before the birth, resulting in no improvement of a severe exophtalmos. The infant showed signs of intrauterine thyrotoxicosis (tachycardia, emaciation, advanced bone age) and was delivered after a gestation of 33 weeks. He presented immediately severe thyrotoxicosis and heart failure, which prevented the undertaking of an exchange transfusion. Under iodine treatment, the symptomatology subsided slowly

Extremely high values of LATS were found in the serum of the mother and of the infant. The half life of detectable LATS in the serum of the infant was about 20 days. TSH level was originally low in the infant and rose under Lugol therapy These findings support the view that LATS of maternal origin is the cause of the temporary thyrotoxicosis of the infant.

Date	Age (days)	PBI µg %	T uptake, %	TSH, mU/ml*	LATS, %/0.5 ml serum
Values obtained in case 2					
25 10.66	2	13 1	36 1	0 14	518 ± 104
15 11 66	23		13 4	0.18	230 ± 37
25.11 66	33			0.23	130 ± 34
20 12.66	58	40.7	22.5	0.35	90 ± 25
Values obtained in the mother of case 2					
5.11 66		5 5	10 1	0.37	1015 ± 202
5 1 6		2.0	23 9	0 12	1060 ± 296

The mother received Prednisone for month before 5 1 67
Radioimmunoassay Method of M Kenzie, *Endocrinology* 63·372, 1958 (Serum activities at 9 h).

M. Pierson, J G Sapeller & J Duheille (Clinique Infantile Universitaire, Nancy). *Neonatal goiter with thyroid antibodies*

Two cases of neonatal goiter are reported.

I. Patient, a boy of 3780 g was born by normal delivery from an euthyroid but goitrous mother

Thyroid enlargement in the newborn was medium. Radioiodine uptake was 79% at 24 hours, but release was fast. Thiocyanate and D.I.T test was negative. The child showed slight symptoms of thyroid deficit: hirsutism, slowness of bony maturation, generalised condensation of the skull, low calciuria, iodine levels in the plasma, by neutronic radioactivation technics, were rather low 5.4 µg for thyroxin-iodine.

Immunological studies (hemagglutination and immunofluorescence) showed presence of anti bodies in sera both from the mother and the newborn

In the newborn antithyroglobuline + anti cytoplasmatic + no antinuclear antibodies. Three months later antibodies were not found.

In the mother's serum: antithyroglobuline ++ anticytoplasmatic + and again no antinuclear antibodies. Three months later the antibodies persisted.

II. Patient, a boy of 3960 g, was born by normal delivery from a goitrous but euthyroid mother

The goiter in the newborn was large. Iodine uptake was 23% in the first hour 44% after 24 hours and 39% on the second day

The newborn showed no clinical or radiological signs of hypothyroidism. Immunological studies:

Newborn: thyroglobuline + cytoplasmatic neg., nuclear antibodies neg. Three months later antibodies disappeared.

Maternal blood thyroglobuline + cytoplasmatic + nuclear antibodies neg. Antibodies persisted three months after delivery

The usual causes of neonatal goiters such as endemic iodine deficiency maternal ngestion of antithyroidal components, maternal hyperthyreosis, hereditary defect of hormonogenesis could be excluded.

Simultaneous finding of the same antibodies in the mother and in the newborn uggests an immunological process as the ca se of the goiter The possibility that one of the ntibodies or responds to LATS is uggested

M. Friedman, G Hatcher & L. A \ tson (Department of Paediatrics and Medica nits, University College Hospital, London) *natal hypomagnesaemia and hypocalcemia*

A reduction in the circulating divalent c tion cal cium is recognized to cause convulsion n the immediate neonatal period. Hypomagnesaemia has been reported in a variety of patholo ical conditions associated with gastrointestinal lo of magnesium 0.2 mg/100 ml) and hypocalcaemia temporary hypomagnesaemia producing con ul sions in a neonate

We have recently studied a male infant who presented with generalised convulsions at 3 weeks of age due to hypomagnesaemia (plasma magnesium 0.2 mg/100 ml) and hypocalcaemia (plasma calcium 5.5 mg/100 ml) Increasing the magnesium intake to six times the normal re quirements raised both the plasma calcium and magnesium to nearly normal levels and cont olled the fits.

The abnormality of magnesium metabolism has persisted during a period of 20 months of follow up. Attempts to withdraw magnesium supplements led to fall in both plasma magnesium and calcium levels and to a recurrence of fits. Calcium and magnesium balance studies carried out at intervals duri g the period of follow up will be presented.

We believe that this patient has a primary inherited disorder of magnesium metabolism and probably represents a new disease entity The nature of the defect remains uncertain, but the evidence is consistent with an isolated malabsorption of magnesium from the gastrointestinal tract.

The patient is probably identical with the patients described by Paunier *et al* (1965) in *Abstracts of American Paediatric Research Society* and the patient described by Salet *et al.* *(1966)*

Otfrid Butenandt (Universitätskinderklinik München tr by Dietrich Knorr). *Serum-enzyme act ty in hypothyroidism*

In hypothyroid children the activity of GOT GPT MDH and LDH in serum is h gher than in healthy euthyroid children No difference could be found between euthyroid children having goiter and healthy children. Under adequate treat m nt, serum-enzyme-activity of hypothyroid ch l

		GOT (IU)	GPT (IU)	MDH (IU)	LDH (IU)
Healthy children	30	14 1 ± 4 7	6.5 ± 2.3	78 ± 33	163 ± 73
Euthyroid and goiter	10	11 0 ± 4.2	6.2 ± 2.3	87 ± 47	140 ± 59
Hypothyroid children	11	24.3 ± 6.8	10.1 ± 4 1	117 ± 38	215 ± 61
Treated hypothyroid	22	11 9 ± 2.9	6.1 ± 2.3	92 ± 54	149 ± 47

dren reaches values, comparable to those of healthy children.

Since it is found (1) that the activity of mitochondrial dehydrogenases in rats is lowered under experimental feeding of thyroxine one can think of a higher consumption of the enzymes in the faster metabolism. Thyroxine increases the metabolism under direct influence on the oxydative phosphorylation (2). Higher consumption in euthyroid than in hypothyroid individuals would explain the difference of the serum-enzyme-activity On the other hand an influence of the thyroid hormone on the cell membrane (change of permeability) or on the enzyme-activity itself must be considered. Most likely the change of the cell membrane permeability is the underlying cause.

M Cruz, J M. Francés, J M. Lloret & J Sabater (University Department of Pediatrics, Barcelona): *A comparative study of the achilles tendon reflex test (photomotogram) cholesterol and PBI in hypothyroidism*

A comparative study of cholesterol PBI and photomotogram as diagnostic tests for hypothyroidism was performed in 10 children with congenital aplasia or hypoplasia of the thyroid gland. Without treatment the three tests were abnormal. After administration of progressive doses of thyroxine the photomotogram normalised first and cholesterol and PBI significantly later After treatment was stopped, PBI and cholesterol detected first the hypothyroid state, while the photomotogram became abnormal much later

We conclude that the photomotogram is a less sensitive test than cholesterol and PBI in hypothyroidism. But because of the frequency with which it is abnormal in children with hypothyroidism and the speed and simplicity with which it can be carried out, it must be considered a diagnostic test of clinical interest.

J M Abraham & A. Russel (Queen Elizabeth Hospital for Children, London intr by Zvi Laron): *Cornelia de Lange syndrome (endocrine studies)*

Multiple endocrine hypofunctions have been reported previously in this syndrome. To explore this eight classical cases (2 girls and 6 boys) were investigated.

Thyroid function. Basal P B.I. and T.3 resin uptakes were normal, the former nearly doubled following T.S.H. stimulation. Initial ^{131}I uptakes were within the normal range. The 4-hour uptake at 16 hours and 5 days after T.S.H., showed a pattern of secondary hypothyroidism in three patients, primary hypothyroidism in two and normal in three. Direct estimations of T.S.H. were not done.

Hypothalamic pituitary-adrenal axis: There was normal response to intravenous insulin induced hypoglycaemia (0 03 units/kg) in 7 patients, with a subnormal response in one. Though the basal levels of urinary 17 K.G steroids were low there was a normal threefold and twofold increase following 8-hour intravenous A.C.T.H. (25 units) and 4-hour metopirone (40 mg/kg) respectively Fasting plasma cortisol levels were high, with a markedly greater than normal response within the first hour following 10 units intramuscular injection of A.C.T.H. or lysine vasopressin. Plasma human growth hormone assays were within the normal range.

Selective T.S.H. deficiency in those showing secondary hypothyroid ^{131}I uptake pattern, or hypothalamic abnormality is presumed.

M I New & R. K. Peterson (Department of Pediatrics and Medicine, Cornell University Medical College, New York, N Y): *A new form of congenital adrenal hyperplasia*

A new form of adrenal hyperplasia in a 12 year old boy is being described. The unique features

of this syndrome are: (*a*) classical signs of hyper aldosteronism i.e. benign hypertension, hypokalaemic alkalosis, low plasma renin, expanded plasma volume and hyperaldosteronism, unresponsive to sodium restriction or sodium administration (*b*) low normal plasma levels of cortisol, corticosterone but elevated plasma aldosterone levels; (*c*) elevated plasma ACTH levels, (*d*) sluggish response to ACTH administration of the baseline low normal urinary free cortisols, 17-hydroxycorticoids and 17-ketosteroids, pregnanetriols and pregnanediols (*e*) normal response of plasma testosterone to chorionic gonadotrophin administration (*f*) decrease of aldosterone production to low levels and marked fall in elevated blood pressure following treatment with glucocorticoids, (*g*) after 4 months of continuous therapy with prednisone, blood pressure has remained normal and the aldosterone response to sod um restriction and sodium administration returned to normal. Extensive metabolic studies and steroidal determinations utilizing the double isotope dilution derivative technique indicate that an overproduction of an ACTH dependent adrenal steroid (or steroids) seems responsible for the syndrome On the basis of steroidal data obtained, a partial 17-hydroxylase defect in the adrenal and not the gonad appears to explain the syndrome best. This form of hypertension is noteworthy because it is alleviated by medical treatment and may be misdiagnosed as primary hyperaldosteronism.

Baseline values

Urine aldosterone	*Plasma aldosteron*
17–26 μg/d	4 m μg%
(normal 14)	(normal 3–15)
Plasma ACTH	
1.5 m unit%	
(normal 0.3–0.7)	

Avinoam Kowarski, Alex Russell & Claude J Migeon (Hadassah University Hospital and the Johns Hopkins School of Medicine Intr by Zvi Laron): *Salt losing tendency in the hypertensive form of congenital adrenal hyperplasia (C.A.H.) induced by treatment uth glucocorticoids*

Parallel sodium balance and aldosterone secretion rate (A.S.R.) studies were carried out on four patients with the hypertensive form of C.A.H. (The 11-hydroxylase insufficiency). Three of these patients manifested a severe form of the disease, whilst the fourth was a milder case.

The three severe cases failed to adequately increase their A.S.R. when on a low sodium diet, and consequently went into negative sodium balance when on treatment with cortisone. In one of these a period of low sodium intake had precipitated a hyponatremic, hyperpotassemic crisis.

It is suggested that in some cases it may be necessary to supplement glucocorticoids treatment with mineralocorticoids, in the management of severe cases of the hypertensive form of C.A.H.

H. Zimprich & D Gupta (The Mautner Mark hofsches Kinderspital Vienna, and the Institute of Child Health, University of London, London): *Urinary androgens and corticosteroids in three cases with salt loosing synd ome*

Individual C 19 and C 21 steroids of the urine of 3 cases with salt loosi g syndrom of the new born are examined and are found to have typical different steroid excretion patterns. One child is shown to have a 21-hydroxylation defect, the second a 3beta-ol-dehydrogenase defect, the third an 18-hydroxylation defect.

The biochemical f ndings in the urine permit a clearcut differential diagnosis between these enzyme defects even without the block being complete. The clinical picture of the patients is correlated to the biochemical findings, showing maximal virilisation in the 21-OH defect. Pathognomonic substances are pregnene-derivatives in the 3beta-ol defect, pregnanetriol in the 21-OH defect and an elevation of corticosterone and its metabolites in the 18-OH defect.

Herman J Degenhart, Henk K. A. Visser (Dept. of Paediatrics, University of Groningen) Elly M. Desmit (Dept. of Paediatrics, University of Ut echt) & Willem S Cost (Dept. Int. Med. Red Cross Hospital, The Hague) *Mineralocorticoid excess i two brothers ith dwarfism and hypok laemic lkalosis*

Using isotope dilution methods secretion rates (SR) of cortisol (F), aldosterone (ALD) desoxy corticosterone (DOC) corticosterone (B) and 18-hydroxycorticosterone (18-OHB) were determined in two brothers (I. 10 8/12 yr II 7 6/12 yr)

SR	Pat. I 112.5 cm, 0.80 m				Pat. II 95.5 cm, 0.60 m²				Control, 12 yr, 1.27 m²	
mg/24 hr	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)
DOC	1.2	0.99	—	—	0.64	0.53	—	—	1 4	0.38
B	3.7	1.3	6.1	0.51	3.3	1.3	4 1	0.32	1.2	1.5
18-OHB	0.14	0.67			0.20	0.96			0.20	0.51
ALD	0.65	0.23	2.5	1 7	0.50	0.35	0.45	0.30	1.21	0.32
F	11 7	7 0			8 8	6.8			7.5	9 1
Renin[a]	11100	2450	10600		2450	1350	2750		24.5	12 (U/L plasma)

By D J J Brown, St. Mary Hosp. London.

with dwarfism, chronic hypokalaemic alkalosis and normal blood pressure. Both demonstrate hyporesponsiveness of blood pressure to infused angiotensin and hyponatraemia on low Na diet.

Periods (1) and (3): low Na, high K intake (20 and 140 mEq/ 4 hr) period (2): high Na, high K intake (120 and 140 mEq/24 hr) period (4) low Na, high K and dexamethasone, 3 × 0.5 mg/d, SR on 4th day

Urinary excretion pattern of F B, ALD and metabolites was in agreement with SR data. Findings indicate relatively small mineralocorticoid excess (mainly B) to highly elevated renin concentrations, and some regulatory control of renin-angiotensin-mineralo-corticoid system. The primary defect of the syndrome ("Bartter's syn-) is still unknown, but might be a renal lar defect in the reabsorption of sodium.

Walter Teller & Gertrud Mürset (Departments of Pediatrics, University of Marburg, and Zurich) *Steroid excretion patterns in precocious puberty during long-term treatment with gestagens*

In previous reports it was shown that gestagens partially suppress precocious sexual development (Schoen, 1966) Only in one study detailed analysis of the urinary excretion of individual steroid metabolites was performed in three children during treatment of idiopathic isosexual precocious puberty with 6alpha-methyl-17acetoxy-Δ⁴-pregnene-3 20 dione (Dep-Provera®) (MAP) (Gupta and Zimprich, 1966). The results did not reveal significant changes of steroid excretion patterns in the course of treatment with MAP

Six patients (4 boys and 2 girls, 1 ³/₁₂–6 years old) with precocious puberty of the idiopathic type (except one boy who had a brain tumor) were treated by injection of MAP 50–100 mg every 10–14 days, for periods from 7 to 1 months.

At various intervals, 24 h-urines were collected for the determination of C 19 and C 21 steroids according to a method published elsewhere (Teller 1967). The following ten steroid metabolites were individually determined. androsterone (A), dehydroepiandrosterone, etiocholanolone (E) 11 hydroxyetiocholanolone, 11-hydroxyandrosterone, 11-ketoetiocholanolone, 11-ketoandrosterone, tetra-hydrocortisol (THF), allo-tetrahydrocortisol (allo-THF) tetrahydrocortisone (THE)

In accordance with the failure of suppression of bone age and height age by MAP the steroid excretion patterns remained essentially unchanged Even after 21 months of therapy the 5 alpha/5 beta ratios of 11-deoxy C 19-steroids as well as the percentages of allo-THF were elevated above normal ranges, which is typical of precocious puberty and also occurs in prepuberal children after single application of testosterone

From our results it is concluded that MAP administration fails to inhibit efficiently and/or persistently the secretion of gonadotrophins. They continue to be secreted in amounts sufficient for the stimulation of endogenous androgen production.

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J R. Bierich & W Blunck (Universitäts-Kinderklinik Hamburg-Eppendorf): *On the relationship between idiopathic precocious puberty and so called premature thelarche*

Report on 18 girls with idiopathic precocious puberty (group A) 4 girls with temporary symptoms of precocious puberty (group B), 18 girls

with "premature thelarche (group C). Group A the following criteria demonstrated the precocious onset of gonadarche and adrenarche: pos. FSH excretion (in 50%), increased excretion of oestrogens, pos. vaginal smears elevated urinary 17-CS, expecially 11 Deoxy 17-CS. Group C: 9 of 13 girls in whom vaginal smears were taken showed a marked oestrogenic effect. Two determinations of urinary oestrogens yielded amounts around 20 μg per day In 1 girl we demonstrated an excretion of gonadotrophins of ~6 MU There was neither an increased excretion of 17-CS nor an advanced height or bone age. In these cases we suggest a temporary precocious gonadotrophic stimulation of the gonads without precocious adrenarche Group B kept a position between group A and C. In 2 cases we saw periods of precocity lasting a few months which included symptoms of gonadarche and adrenarche. Two other girls exhibited an early menarche accompanied by high urinary oestrogens without any signs of premature adrenarche. The symptoms disappeared after 3 and 10 months, respectively I these 2 cases the stimulation of the ovaries apparently exceeded that which is normally seen in common premature thelarche. Usually the gonadotrophic stimulation seems to be too weak and of too short duration as to bring about vaginal bleedings.

Dietrich Knorr (Universitäts-Kinderklinik, München): *Gas-liquid-chromatographic studies I boy with excessive gynecomastia*

Gas-liquid-chromatographic studies of the steroid-excretion were performed in 7 boys with excessive gynecomastia. Histologically a proliferation of the ductus lactiferi were found in these patients, but no proliferation of the excreting cells.

Since progesterone stimulates the growth of the ductus lactiferi in animals, we determined again the urinary excretion of pregnandiol, the main metabolite of progesteron. Gas chromatographic values are significantly lower than the values obtained with the method by Klopper Boys with pubertal gynecomastia do not excrete more pregnandiol than do normal boys in puberty But the boys with gynecomastia regularly excrete slightly more Pregnan-3a,17a,20a-triol. Mean value is about 700 μg/d. In addition, a large amount of the Trimethyl-allyl-ether of a steroid is found constantly in gaschromatograms on P XE 60 which has a retention time of 1 18–1.2.. compared with Trimethyl-silyl-oestron = 1 00.

Werner Blunck & Jürgen R. Bierich (Universitäts-Kinderklinik, Hamburg-Eppendorf): *Urinary excretion of the A-ketolic metabolites of cortisol and corticosterone and the 11-oxy and 11-desoxy 17-ketosteroids in childhood*

After conjugate extraction, glucuronidase-sulfatase hydrolysis and solvolysis, purification of the extracts on florisil, a paper-chromatography in two systems and in some fractions purification by thin-layer-chromatography is performed. The quantitative estimation has been done photometrically in vitro by blue tetrazolium- or Zimmermann-reagent.

In 35 normal children of different age no qualitative changes in corticosteroid excretion during puberty were found, there were only quantitive changes according to body surface. Especially there were no statistically significant differences in the allo-THF-ratios. The excretion of corticosterone metabolites in the age group 4–6 years was rather high.

If only glucuronidase hydrolysis has been used, there was a significant lower yield of allo-THF before puberty (20 cases)

The difference of the allo-THF recovery after different hydrolytic procedures might be explained by the presence of allo-THF-sulfate in the urine of the children. After extraction of steroids split by glucuronidase, allo-THF could be detected in extracts treated by solvolysis, but the amount was rather low and not sufficient to explain the difference completely

The values for seven 17-ketosteroids were in the range already published by other authors.

In precocious puberty the 5 /5β-ratio was within the range (mean $\pm$ 2 sigma) of the chronological age group. The values in some diseases of the adrenal cortex (M Cushing, adrenal carcinoma and 11-hydroxylase deficiency) are discussed

William Hamilton & G C Arneil (Department of Child Health, University of Glasgow) *Urinary metabolites of prednisolone during treatment of nephrosis*

I our unit we have been treating for several years, cases of nephrosis with prednisolone in the

following dosage: 60 mgm, 40 mgm, 10 mgm, 5mgm daily each for 10 days so that a patient received a 50–60 day course. The steroid is there after "tailed off".

It is our observation that between the 10th and 14th day most patients have a spontaneous diuresis and their urine becomes albumin free. Only rarely do we require to add a diuretic in which case it is to alleviate distressing dependent oedema.

The urinary excretion of the administered prednisolone was investigated in these cases. During the period of administration a sodium fluorescent steroid (indicating a possible Δ^4 configuration) is excreted. This substance is not a product of the hydrolytic procedure in vitro nor is it recognised as an *in vivo* metabolite of a Δ^1 -steroid.

This substance was not detected in the urine of non-nephrotic patients who are on prednisolone and its full identification and quantitation might give significant information regarding the nature of the steroid disturbance in nephrosis. Evidence relating to this substance will be given.

Eleazar Shafrir (Laboratory of Clinical Biochemistry Hebrew University–Hadassah Medical School and Hadassah University Hospital, Jerusalem intr by Zvi Laron): *Hormonal aspects of adipose tissue metabolism*

...e mode of action of principal hormones af... g the metabolism of adipose tissue will be ...ortly reviewed. Against this background, specific problems of regulation of fat metabolism in the fetal and neonatal state will be discussed. There appears to be no significant direct connection in fat metabolism between the maternal and fetal organism, since neither free fatty acids and their albumin carrier nor macromolecular lipoproteins pass freely through the placental barrier. Lipid content of fetal blood is very low and fetal adipose tissue is not well developed up to a short time before birth. The adipose tissue exhibits an avid glucose uptake and low fat release, indicating that the equilibrium is in favour of triglyceride deposition and carbohydrate metabolism is the main energy source. Soon after birth the importance of fat metabolism rises, sometimes in an abrupt manner possible in association with neonatal hypoglycemia. The ensuing counter regulatory measures result in the release of free fatty acids from adipose tissue. The short term compensation necessary for survival seems to be brought about by activation of the nervous system and of catecholamine release followed by the long term influence of growth- and other hormones. These responses of adipose tissue appear to represent the first hormonally compensated emergency in life.

Václav Melichar Milan Novák & Petr Hahn (Institute Mother and Child Care Prague): *Peculiarities in lipid metabolism of newborns from diabetic mothers*

In full term healthy newborn infants endogenous lipid reserves are mobilized. The levels of FFA, ketone bodies and glycerol rise in the blood and FFA and glycerol content increase in adipose tissue. In newborns from diabetic mothers the rise in the blood level of FFA is much smaller, ketone bodies remain at a lower level than in full term newborns during the first postnatal week. These differences in lipid utilization evidently are due to the high level of insulin in the blood of newborns of diabetic mothers but perhaps also to the higher liver glycogen content. The second possibility is supported by the fact that in dysmature (small for date) newborns the rise in FFA and ketone blood levels is highest while their liver glycogen content is lowest.

Olav Trygstad (Rikshospitalet, University of Oslo): *A human pituitary lipid mobilizing factor (LMF)*

Clinical observations (congenital generalized lipodystrophy lipoatrophic diabetes, panhypopituitarismus, Fröhlich's syndrome) and endocrinological research (Anselmino and Hoffmann's "Fettstoffwechselhormon Seifter and Bader's Lipid Mobilizer Rudman's "fraction H" Astwood's Peptide I" and Peptide II" Li's "lipotropin Chalmer's F t Mobilizing Substance") have provided evidence for the existence of a specific pituitary hormone regulating release of nonesterified fatty acids (NEFA) from depot fat.

From human pituitary glands a lipid mobilizing factor (LMF) has been extracted, and purified by Sephadex gel filtration and DEAE-cellulose chromatography which yielded two lipolytic fractions (dLMF I and dLMF II). Removal of *LMF* from the pituitary extract almost nullified the

adipokinetic, hyperglycemic and rabbit serum calcium lowering effect of growth hormone and pituitary gonadotropins. Furthermore the molecular weight of HGH was reduced from 25 000 to 20,000, and disc electrophoresis gave two instead of three bands. It has been discussed whether the LMF might be the diabetogenic factor of growth hormone or a lipotropic hormone of itself

The dLMF I has one band on electrophoresis and a mol.w determined by ultracentrifugation in the range of 2500 dLMF II has two bands on electrophoresis and a mol.w in the range of 4000.

Both are potent lipotropins in rabbit and human fat *in vivo* (in doses of 0.05 mg and 5 mg respectively) and in vitro (in doses of 0.00001 and 0.01 µg/ml). The activity in mouse and rat fat is questionable. Both give a prolonged hyperglycemia. The dLMF II has a potent rabbit serum calcium lowering effect. In high dosage it is calorigenic and toxic in the rabbit, may lower serum calcium below 3 mEq/l, and the rabbits may die with convulsions.

J Fernandes & N A. Pikaar (Wilhelmina Childrens Hospital, University of Utrecht and the Central Inst. for Nutr and Food Research, Zeist; intr by Robert Steendijk): *The influence of dietary carbohydrat and fat on the lipemia and the subcutaneous fat of children with liver glycogen disease*

The lipemia commonly found in children with liver glycogen disease was studied in 2 children with different types of enzyme defect. The lipemia appeared to be hyper-beta-lipoproteinemia with concomitant normal to low alpha-lipoprotein levels. The lipemia was especially prominent during a normal cow's milk formula with extra starch and glucose added. Isocaloric replacement of most of the carbohydrates by corn oil or olive oil had a striking effect on the fatty acid composition of the serum lipids. Moreover the levels of total fatty acids, cholesterol and beta-lipoprotein showed a distinct decrease.

The favourable effect of a diet high in corn oil was undone by the introduction of coconut fat.

Elimination of the unsaturated fat and reintroduction of carbohydrates on an isocaloric basis caused the return of the original serum lipid pattern with a predominance of saturated and monoenoic fatty acids. Moreover it caused an increase of beta-lipoprotein levels.

On account of the data obtained during these experiment it seems probable that the lipemia in liver glycogen disease is caused by a carbohydrate induced hepatic hyperlipogenesis.

Serial studies of adipose tissue were feasible by aspirating small samples of subcutaneous fat according to the method of Hirsch at the beginning and at the end of these periods when the dietary fat was changed or fat and carbohydrates were interchanged. The fatty acid composition of this tissue was investigated. The linoleic acid content was found to be strikingly influenced by the linoleic acid content of the dietary fat.

Gaston R. Zahnd & David Klein (University of Geneva, intr by Zvi Laron): *Insulin and growth hormone secretion during impaired glucose tolerance in Laurence-Moon-Bardet-Biedl syndrome*

The mechanisms responsible for the severe adiposity encountered in patients with Laurence Moon-Bardet-Biedl's syndrome are unclear although the occurrence of decreased glucose tolerance has been repeatedly reported.

In this study simultaneous measurements of immunoreactive insulin and growth hormone, of glucose and free fatty acids were performed during a rapid intravenous glucose load (0.5 gm per kg) in two brothers presenting the Alström–Hallgren type of the LMBB-syndrome. Despite the presence of diabetic k values (1.20 and 1.07) the basal concentrations of plasma free fatty acids were within normal limits. Markedly elevated fasting insulin levels (144 and 230 µU per ml) were followed by a delayed and exaggerated pancreatic insulin-release after glucose. In contrast to the findings in relation to the insulin secretion, a significant depression of plasma growth hormone levels (range 0 to 3.1 ng per ml) was observed in both cases.

The excessive insulin response found in this particular form of obesity does not differ qualitatively from the hyperresponse of insulin to glucose in obese maturity-onset diabetics and the reduced growth hormone secretion could correlate with the assumption of a congenital hypothalamic lesion in the LMBB-syndrome.

Hans Helge, Bruno Weber, Gottlieb Sihombing & Egon Werner (Kinderklinik, Nuklearmed. Abt. Freie Universität, Krankenhaus Wedding, Berlin):
Effect of tolbutamide on FFA and insulin in normal and obese children

Fasting levels of FFA and plasma insulin as well as their response to a tolbutamide stimulus differ in normal and obese adults (1). In this study the effect of i.v. injection of tolbutamid (20 mg/kg) was studied in 10 obese children and adolescents. FFA were determined according to Trout *et al.* (2), insulin by a minor modification of method C of Hales and Randle (3). Both, FFA and insulin, were increased in the fasting state. As in normal individuals, tolbutamide injection provoked a prompt decrease of FFA and an increase of insulin concentration in plasma. However the extent of his reaction, in spite of a similar blood sugar response, was more pronounced in the obese group. Also FFA returned slower to the fasting values and the period of elevated plasma insulin was prolonged.

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Z. Laron, M. Karp, A. Golomb, A. Kowadlo-bergeld, M. Dudek & D. Harel (Pediatric Clinic and Endocrine Service, Rogoff Wellcome Res. Inst., Beilinson Hosp., Pediat. Dept., Tel Aviv Univ. Med. School):
Lipolytic effect of epinephrine and growth hormone in obesity. In vivo and in vitro studies

Ten medium obese boys, eleven medium obese girls and 5 normal boys aged from 7 to 17 years were examined for their response of plasma FFA, glycerol, sugar and growth hormone (GH) after the administration of epinephrine (i.m.) or insulin (i.v.). In 12 of the patients suprailiac adipose tissue was obtained by biopsy and the release of glycerol from these samples was studied after *in vitro* incubation with epinephrine and human GH. Control studies were performed on adipose tissue obtained at 14 gynecological operations. Additional studies included FFA mobilization after i.v. administration of HGH and measurement of subcutaneous fat tissue changes during prolonged i.m. therapy with HGH of patients with pituitary insufficiency.

The following results were obtained:

The obese boys and girls had a tendency for higher fasting FFA and glycerol levels than the control and showed a greater increase in plasma FFA, glycerol and blood glucose to epinephrine than the control subjects. After ingestion of insulin there was a drop of plasma FFA and glucose and a slight rise in glycerol in all groups. There was no marked difference between the groups and mean values tended to return to initial values at two hours.

The injection of insulin elicited a marked rise of serum HGH in normal controls with a peak at 60′ in most instances. In the obese patients two trends were observed: some had a normal rise with a faster decrease than the controls, other patients had a subnormal GH stimulation. Epinephrine had little effect on plasma GH in all groups.

In vitro both epinephrine and HGH caused lipolysis of the human adipose tissue samples in most patients. Intravenous administration of HGH after an overnight fast induced marked elevation in pituitary insufficiency but much less so in obesity. Prolonged administration of HGH induced in 4 out of 5 instances a reduction of the subcutaneous fat tissue.

François Girard, Michel Binoux, Micheline Combourieu & Pierre Mozziconacci (Hôpital Trousseau, Paris, intr. par Raphael Rappaport):
Variations circadiennes de l'activité corticotrope du plasma

L'ACTH plasmatique a été mesuré par une méthode biologique à diverses heures du nycthémère.

Chez 4 sujets normaux on observe une variation cyclique du taux de l'ACTH circulant parallèle à celle du Cortisol dont les caractéristiques sont bien connues. Dans la soirée ACTH et Cortisol sont à leur niveau minimum.

Les arguments suivants plaident en faveur d'un cycle autonome de l'ACTH tenant sous sa dépendance le cycle du Cortisol:

— L'abaissement artificiel du Cortisol à l'aide de la Métopirone ne provoque pas de montée de l'ACTH dans la soirée alors qu'une réponse hypophysaire est observée le matin.

— Dans l'hyperplasie virilisante congénitale des surrénales le déficit de la synthèse du Cortisol entraîne une élévation de l'ACTH plasmatique dont le taux mesuré le matin se situe à deux fois et demi au-dessus de la normale et la réponse hypophysaire à la Métopirone, d'une amplitude considérable, conserve cependant les caractéristiques circadiennes observées chez le sujet normal.

— Chez un sujet atteint d'une maladie d'Addison modérée, le Cortisol reste bas et stable alors que l'ACTH plasmatique présente des variations cycliques.

— Par contre dans la maladie de Cushing les cycles du Cortisol et de l'ACTH plasmatique sont perturbés et la réponse hypophysaire à la Métopirone ne se fait plus dans le deuxième partie de la nuit.

De l'analyse de ces faits, il résulterait que la libération active de l'ACTH et sa régulation par le taux du Cortisol circulant ne se ferait que pendant quelques heures dans la deuxième moitié de la nuit. Pendant le reste du nycthémère l'hypophyse et la corticosurrénale ne présenteraient plus qu'une activité résiduelle.

R. Rappaport (Hôpital Enfants-Malades, Paris): *The thyrotropin stimulation test in hypopituitary dwarfism: evidence for limited thyroid reserve*

Thyroid function was evaluated in 28 cases of hypopituitary dwarfism by measurement of the "Hormonal Protein Bound Iodine" after fractionation on Resin Dowex 50 column and radio-activation analysis of the stable iodine. Normal values for children are 5.02 ± 0.95 $\mu g/100$ ml plasma (90 cases). Children with pituitary insufficiency showed mean value of 3.4 ± 0.9 $\mu g/100$ ml plasma (range 1.7–8.1). There is a large overlap with the group of partial primary thyroid insufficiency.

Thyrotropin stimulation was carried out by a daily injection of 100 units Heyl-Laqueur during seven days and hormonal protein bound iodine measured at the end of this period. The increase was 7 ± 1 $\mu g/100$ ml in normal children and below 4.4 $\mu g/100$ ml in 11 out of 18 hypopituitary dwarfs investigated. The reason for a limited thyroid reserve is discussed: the long duration of the disease does very likely play role as demonstrated in three cases.

Reference

R. Rappaport, D. Comar, O. Cachin & P. Royer: L'insuffisance thyréotrope. To be published in *Arch. F. de Pédiatrie*.

Etienne E. Joss, Klaus A. Zuppinger, Gaston R. Zahnd & Ettore Rossi (Pediatric Department of the University of Berne and the Medical Policlinic of the University of Geneva, intr. by Gertrud Mürset): *Evaluation of diagnostic procedures in dwarfism*

The following diagnostic tests for growth hormone (GH)-deficiency were performed in nine children with marked retardation of growth and skeletal maturation.

(*a*) Plasma-GH level during insulin induced hypoglycemia.
(*b*) Insulin tolerance test.
(*c*) Influence of GH-therapy on insulin tolerance test.
(*d*) Influence of two different doses of GH (2 and 4 mg/m2/day) on nitrogen balance.
(*e*) Effect of GH-therapy (10 mg/m2/week for 6 months) on growth rate.

Considering the results of all these tests combined, without giving priority to one of them, the nine children could clearly be divided into two groups, namely dwarfs with and without GH deficiency.

The *diagnostic value* of the different tests can be evaluated as follows:

The level of plasma-GH during insulin induced hypoglycemia and the growth response during the first six months of GH-therapy are the best diagnostic tools for differentiation of GH-deficient and primordial dwarfs. Nitrogen retention on GH is greater in GH-deficient dwarfs, but there is no clear cut separation between the two groups.

The insulin tolerance test as judged by the recovery of glucose from it nadir as well as the influence of GH on the glucose recovery is of little diagnostic value.

G. A. Brown, Douglas Hubble & P. H. W. Rayner (Institute of Child Health, University of Birmingham, Birmingham, England): *The HGH nitrogen retention test in the diagnosis of hypopituitarism in children*

At the Copenhagen meeting in 1965 the results of the HGH Nitrogen Retention Test were re

ported in 13 children of short stature, four of whom were suffering from hypopituitarism. This investigation has now been extended to 26 children of small stature, 15 of whom had been diagnosed as suffering from hypopituitarism. These 15 children had growth hormone levels below 10 m g/ml (below normal values in childhood by the method in use) during insulin induced hypoglycaemia. The hypopituitary children had more than 25% nitrogen retention and more than 70 mg/kg body weight/24 hours during the five day period of HGH administration (10 mg daily) The 11 short children not suffering from hypopituitarism, all showed less nitrogen retention than these values.

Using a shortened version of the test—3 pre-HGH days and 3 HGH days—the diagnostic categories remained unchanged except for one hypopituitary child whose value for nitrogen retention was reduced from 35% to 25%. The effect of HGH on urine urea nitrogen in single specimens collected over the 24 hours for 3 days will be reported for eight children.

A. Pertzelan & Z. Laron (Pediatric Metabolic and Endocrine Service, Rogoff Wellcome Med. Res. Inst., Bellinson Hospital Dept. Pediatric, Tel Aviv Univ Med. School)· *Genetic aspects of pituitary insufficiency with high concentrations of cti e growth hormone*

of 60 patients with pituitary insufficiency der our care, 25 belonging to 15 families have a pathological genetic background. These patients can be devided into 4 groups. (*a*) several afflicted siblings in the same family (16 patients) (*b*) afflicted cousins (⁁ patients) (*c*) single child product of parents with high degree of consanguinity (4 cases) and (*d*) child of affected parent (1 case).

With the exception of one girl, with consanguine parents from Roumania, all families originate in Iraq Yemen, Algeria and Morocco. All these children resemble each other· they are very short, have small acre, well developed subcutaneous fat tissue, bad teeth, retarded bone age and a high pitched voice (1). They have a tendency for hypoglycaemia and are sensitive to insulin. In spite of these signs and symptoms which are characteristic for growth hormone deficiency all (with the exception of one family) have a high plasma growth hormone concentration, up to 200 ng/ml.

Analysis of the various pedigrees suggests a recessive transmittance for this disease which we consider an inborn metabolic error of growth hormone synthesis.

Reference

1 Laron, Z., Pertzelan, A. & Mannheimer S. Genetic pituitary dwarfism with high serum concentration of human growth hormone. A new inborn error of metabolism? *Israel J Med Sci*, 112, 1966.

Jacob J Frankel (Pediatric Metabolic and Endocrine Service Rogoff Wellcome Medical Research Institute, Bellinson Hospital and Department of Pediatrics, Tel Aviv University Medical School, Petah Tikva)· *Psychological correlates of pituitary insufficiency*

Twenty children and adolescents with pituitary insufficiency were administered a psychological battery consisting of visuo-motor tasks and a standard test of intelligence. The group ranged in age from 8 to 20, and represented three classes of pituitary pathology· 1 Genetic or familial, 2 sporadic and 3 craniopharyngioma cases.

The major findings reflect the following: the visuo-motor tasks proved particularly difficult for all three classes of affected subjects, immediate recall of newly-acquired visual material proved unusually difficult as compared with a normal population intellectual functioning in the verbal (or non-performance) realm proved almost uniformly higher for all three groups, but with particular emphasis upon the genetic familial individuals overall estimate of intellectual functioning revealed borderline or lower scores in all subjects, with none reaching the lower limits of the normal category

These results are taken to indicate a qualitative as well as quantitative factor in the intellectual performance of children and adolescents with a pituitary pathology one that is perhaps best subsumed under a central nervous system involvement.

While these results may be unduly affected by sample size and composition, i.e. socioeconomic and educational levels, they are sufficiently at variance with those of Pollitt and Money and Rosenbloom to warrant more intensive research efforts. Such efforts are imperative from the

standpoint of vocational guidance and rehabilitation.

References

Pollitt, E. & Money J. Studies in the psychology of dwarfism. I. Intelligence quotient and school achievement. *J Pediat* 64 415 1964.
Rosenbloom, A. L.: Scholastic performance of short statured children with hypopituitarism. *J Pediat* 69 1131 1966.

Margaret Davidson (Queen Elizabeth Hospital for Children, London) and Alex Russell (Hadassah University Hospital, Jerusalem) (Intr by Zvi Laron): *Juvenile acromegalo-gigantism and somatotrophic status*

Five examples of juvenile acromegalo-gigantism are presented, one after follow-up for 10 years, another for 7 years. Four illustrate a very characteristic natural history indicated in the previous substantial series of so-called cerebral gigantism. The initial grossly excessive advancement in growth and skeletal maturation is confined to the period from first to sixth years of life, possibly correlated in our cases with a hyperphagic phase, and linked moreover with an incomplete acromegaloid pattern of cranio-facial and extremity development. In addition, these cases displayed impairment of speech maturation and articulation, macroglossia, hypertrophied limb muscle mass, forehead bossing, lumbar lordosis, etc. and also a family history suggesting a genetic basis with "dissociated" clinical elements at least in respect of typical megacephaly heavy facial features, characteristic mental backwardness, general clumsiness of movement, and possibly undue tallness. In one subject (H. F.) otherwise typical, there is no megacephaly and the I.Q. is high (148% Merril-Palmer scale).

Growth hormone (G.H.) assays have been undertaken in all 5 subjects. Raised levels were not found; four subjects had a normal G.H. secretory response to oral glucose and to i.v. insulin. The fifth patient (S. D.) had a slightly subnormal response to both stimuli. Increased insulin sensitivity was noted in 2 cases (S. W. and M. M.). That G.H. measurements were not available during the peak growing phase of these children imposes an important reservation on the interpretation of these results in relation to that phase.

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Kjellman, B., *Acta Paediat Scand*, *54* 603, 196.

Alex Russel (Hadassah University Hospital, Jerusalem) and Margaret Davidson (Queen Elizabeth Hospital for Children, London) (Intr. by Zvi Laron): *High plasma somatotrophins linked to growth acceleration but negative metopiron response in the diencephalic syndrome of emaciation*

Observations upon 16th personal case of this syndrome (1–4). Arrest of weight gain attending abrupt onset of emaciation from 6–8 weeks: earlier than typical emergence from 3–24 months. Contrasting with typically paradoxical normality or even enhancement of appetite, anorexia was dominant from outset, as in some previous early cases (4). Hyperactive, alert and cheerful rather than cachectic. Neuropathic stigmata often absent, but here much earlier than usual, viz. nystagmus, optic disc pallor abnormal increase of head size. Hyponatraemic and hypoglycaemic trends (1–4) reproduced. *Ventriculography*. Deformation of 3rd ventricular floor. *Gamma Scan*. Abnormal and irregular suprasellar isotopic uptake strongly suggesting glioma. *Craniotomy*. Hypothalamic astrocytoma, as in all cases ("fibrillary" in type as in those of rapid early growth (4).

Growth status: At 4 months: +90th percentile, according with initial growth acceleration of most infantile examples. *Somatotrophic Status*: (Also at 4 months). Exceptionally high fasting G.H. range. 228–237 m μg/ml, falling rather than rising as glucose level declined during glucose tolerance test. Likewise, the fall of blood glucose to zero in insulin test evoked no further rise in possibly maxima G.H. fasting level of 228 m μg/ml. *Growth implications*: Results support thesis (2–4) that the underlying anterior hypothalamic astrocytoma, probably congenital, either produces growth-activating or "releasing" factors or frees them from frontocortical inhibition (analogous to postulated role as neoplastic counterpart or prefrontal leucotomy in evoking euphoric elements).

Parallel metopirone/LB Vasopressin/ACTH Tests. Impaired response confined to metopirone,

as reported in 3 previous cases (4), illustrating dependence of test upon hypothalamic as well as pituitary/adrenal integrity and its usefulness in hypothalamic diagnosis.

Glucose tolerance G.H test

Time (minutes)	0	30	60	90	120	180	140	300
B. glucose (mg%)	47	54	78	61	42	40	38	37
G H. (mμg/ml)	237		190		144	181	190	206

Insulin G H response

	0	15	After resuscitation (glucose and hydrocortisone)
B. glucose	15	0	320
G. H. (mμg/ml)	228	222	162

References

1 Russel, A., *Arch Dis Childh*, 26 274, 1951.
2. — *M D. Thesis (Durham)*, 1952.
3 — Vol. 1 1st Internat. Congr Neurological Sc. 1957 pp. 435–438, 1959
4. — XI Internat. Congr Paediatrics, Tokyo, 1965

Dorothy B Villee (Harvard University Medical School Boston, intr by Zvi Laron): *Endocrine abnormalities in two boys with classical progeria*

Two boys, aged 5 and 10 with classical progeria were studied at the Children's Service of the Massachusetts General Hospital About 30 investigators and laboratories in the Boston area collaborated in an effort to discover if these chil-... have any detectable biochemical abnormality ... of the studies revealed results not signifi-...y different from those of normal children. However the following abnormalities were found in both children and to approximately the same extent: low hydroxyproline and high creatine excretion in the urine, and low concentrations of proline glycine, alanine, serine, threonine and glutamic acid in the blood. Collagen extracted from the skin had a very high shrinkage temperature and solubility properties suggestive of molecules with extensive cross-linkage.

Endocrine studies of the two boys revealed no detectable abnormal ty of thyroid or adrenal function. Both boys were relatively insulin resistant, requiring 2–3 time the normal amount of insulin to produce a 50% fall in blood glucose Insulin injected into the boys had *an abnormally short* half-time. Assays for growth hormone showed no detectable hormone either before or after a 50% fall in blood glucose. Administration of human growth hormone on either a short term (3 day) or a long term (4 mo) basis produced no significant nitrogen retention. The boys neither grew nor gained weight.

(Supported by USPHS grant AM 08076.)

J Čížková, M. Ulrichová & L. Růžička (Children's Hospital, University of Prague: Read by title): *Influence of serum of children and adolescents treated for prolonged periods with corticosteroids on the growth of plants*

The use of plants as material for hormonal tests has an old tradition from the ancient Egyptians. Macht and Livingston introduced in 1922 a root growth test for the investigation of the interaction between the physiology of the animal and vegetable kingdoms. Macht demonstrated in a large number of papers that, under standard experimental conditions, sera from healthy persons inhibit the growth of Lupinus albus roots. We have also investigated sera of healthy children and adolescents and have found that the growth of roots of Lupinus albus was increasingly inhibited with sera from individuals of increasing age and height. The resulting curve is very similar to the growth curve of children. The construction of this physiological curve enables us to compare these inhibitory effects with pathological findings. Among hundreds of investigations our attention was drawn to children suffering from diseases treated for prolonged periods with corticosteroids especially with Prednisone. The sera of 16 such children and adolescents were investigated. Comparing the sera of these children and adolescents with controls, we have observed that the inhibitory factor was significantly higher during Prednisone therapy The two distributions were compared by means of the non-parametric statistical test (Wilcoxon test) at 5 p.c. level of significance.

FILM REVIEW

A FILM[1] ABOUT BREAST FEEDING IN SWEDEN

THE SWEDISH MIDWIVES' ASSOCIATION (*Svenska Barnmorskeförbundet*) has presented film on breast feeding, dealing with its physiology its technique and also the prevention and treatment of breast feeding difficulties. The text of the film has mainly been written by Dr Kerstin Adrian of Department II of Obstetrics and Gynaecology in the Sahlgrenska hospital, Gothenburg. The film has been made in co-operation ith Pump AB Einar Egnell, Trollhättan (Mr Einar Egnell and Mr Holger Infeldt).

The film is of an informativ character and mainly intended for use in the training of pupil midwives, pediatric nurses, children nurses and other groups of personnel concerned. Indirectly such film can be expected to stimulate interest in breast feeding.

The decrease in the interest in breast feeding observed in Sweden—and in certain other countries to consider ably greater extent—is viewed with some concern by pediatricians in this country. They have therefore issued serious statement in this question. W feel it is by no means unreasonable to consider lactation as being both useful and essential for babies: breast milk flows for the benefit of the baby

All of us who are engaged in maternal and child care agree on this point. W see how the spirit of the age has influenced breast feeding in negative direction: the slightest difficulty and breast feeding is abandoned. Worry hether the breast milk supply is sufficient, stress, nervous problems cause the same result. This is also the case hen wage-earning omen return to their ork. Bottle feeding compounds hich are being improved continuously also provide strong temptation t cease breast feeding. Further reasons can be stated for the decrease in breast feeding. With very few exceptions, however there is strong conviction within maternal and child care in Sweden that breast feeding shou d be both encouraged and helped. Unfortunately this is not always the case in certain circles in other countries.

The film presented by the Swedish Midwives Association provides excellent support in the propaganda for an increase in breast feeding. Particularly significant in the film is the encouraging advice which can be imparted to mothers hen they become depressed through breast feeding difficulties. It is very obvious that one of the main aims of the film is to provide help through information in all cases here there is real desire to breast feed, and to encourage and convince doubtful mothers and those ho are not fully confident.

The film provides excellent information on the technique and physiology of breast feeding. In clear and easily grasped way—to large extent in animated sequences—it shows the anatomy of the breast, the milk production mechanism and the various factors contributing when the breast is emptied The film allow us to follow mothers in their homes before and after confinement and also during the lying-in period at the maternity hospital. It can be seen how mother-to-be can prepare her nipples for their function particularly hen breast feeding problems are to be expected, for example because of nipples which are too small, flat or inverted. Attention is also paid to the treatment of nipple injuries in the form of cracked nipples, lymphangitis and abscesses. The film is particularly important when it deals with the difficulties encountered by mothers during the period immediately after they hav returned home from the maternity hospital, fatigue, household problems, stress, anxiety for the baby and its nutrition. A clear picture is given of the temptations and risks concerning breast feeding during the first critical period hich can last for one or more weeks after the mother has returned home from the maternity hospital. I provides much advice concerning the care of the nipples and their cleanliness, suitable breast feeding times, rest periods etc.

The final part of the film shows the electric breast pump, the conditions for its correct use and the cleanliness involved. The breast pump in question has been designed as the result of extensiv studies into the physiology of breast feeding and the designer has also cooperated closely with various experts obstetricians pediatricians, midwives, hygienists, bacteriologists, etc the aim being to produce pump with an action that was as correct and natural as possible

Many mothers maintain good lactation with the Egnell breast pump This is particularly true for mothers of premature babies ho can, in many cases, provide their child with their ow milk until the child is large enough to suck directly from the breast itself. Milk donors, who deliver their surplus milk to mother milk centre, also use this pump to empty the breast after their own child has received sufficient quantity Surplus milk provided in this way satisfies the present demand for mother milk from the children clinic at our hospital

It can be mentioned that great interest has already been show in this film from countries outside Scandinavia, and various versions in foreign languages are to be produced A the moment the film is vailable in English, French, German and Dutch

As an instructional film "The Management of Breast Feeding in Sweden ill be xtremely valuable.

Örebro, July 1967 *Olof Brandberg*

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[1] The film—16 mm sound film in colour—has running time of 34 minutes It has been produced by Cinég AB, Gothenburg, Sweden, and can be obtained through Svenska Barnmorskeförbundet, Flestbacken 30 Stockholm 5\ Sweden.

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ERRATUM

In the article by B. Kjellman, "Regional lung function studied with Xe^{133} in children with pneumonia vol. 56, no. 5, the -ray recordings of Fig. 3 (on page 470) and Fig. 5 (on page 471 below) have been interchanged.

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WISKOTT ALDRICH SYNDROME

A Study of 6 Cases with Determination of the Immunoglobulins A D G M and ND

G Berglund, O Finnström, S. G O Johansson and K. L. Möller

From the Department of Pediatrics, University of Gothenburg and he Department of Pediatrics, University of Umeå, and he Blood Center University Hospital Uppsala, S eden

Wiskott Aldrich syndrome is characterized by susceptibility to infections, eczema and thrombocytopenia. It affects males and is transmitted sexlinked recessively (1 6, 31).

Recent studies have suggested a defect in the immunological response, by some authors localized to the immunoglobulins (30) by others to the cellular response (3 9).

Almost all of the performed immunological investigations in Wiskott-Aldrich syndrome have demonstrated some changes in the immune system, but the changes vary in different patients. In the patients reported here almost identical changes in the immune responses were found, namely an increase of serum IgA and IgND level a defect delayed hypersensitivity as could be seen from negative tuberculine test after BCG vaccination, and an increased susceptibility to infections.

CASE REPORTS

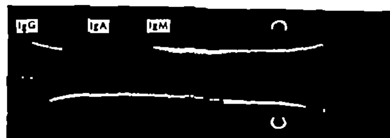
Case 1 B Je., born on Aug. 10, 1961 Second son of healthy mother In the family there is an allergic heredity on the father as ell as on the mother side, but no one, except younger brother (Case 2) has the same disease. There is no known consanguinity in the family

In the neonatal period the patient had melena during weeks. From the age of one month he showed an increased tendency to upper respiratory infections and otitis. Eczema developed at the age of 2 months, shortly after exposion to cow milk. Because of infections and eczema he was admitted to the Children Hospital, Gothenburg, at the age of 5 months. At that time he demonstrated skin hypersensitivity t cow milk. He as treated with milk free diet during some years ith some effect on his eczema. At the age of 8 months generalized lymphadenopathy and hepatosplenomegaly developed. His general condition was poor During the following 4 years he suffered from repeated upper respiratory infections ith pneumonia and atelectasis, caused by Staphylococcus aureus, haemolytic streptococcus and pneumococci. He was treated with tetracyclines and chloramphenicol since he achieved an allergy t sulfonamids and probably to penicillin during his first year of life. His eczema and his general condition improved somewhat during the following years. At few occasions he demonstrated an increased bleeding tendency with haematomas and blood in the stools. This occurred only in connection with severe infections, but his bleeding manifestations never needed any treatment. Twice he had periods with septic fever arthralgia, myalgia and episcleritis. This occurred once after an injection of gammaglobulin, given because of temporary low gammaglobulin level, and once after treatment with oxacillin. At the age of 6 years he died of overwhelming infections; pneumonia with empyema and acute meningitis caused by *Haemophilus influenzae*

Laboratory findings ere: Haemoglobin 7.8–11 6 g/100 ml. Reticulocytes 0.3–2.9 %. Leucocyte count 15,000–37,000 with normal distribution of cells except for eosinophilia. Total eosinophil count 2000 to 4000/mm³ Micro sedimentation 40–60 mm. Serum iron 55–70 μg 100 ml. Platelets 20,000–60,000/mm³

Bleeding time 3–6 min. Clotting factors normal. Fibrinogen slightly elevated. Prothrombin consumption pathological. Clot retraction poor No fibrinolytic activity Bone marrow examination revealed normal myelopoiesis except for high content of eosinophils. The erythropoiesis as reduced, the lymphocytes and the plasma cells ere normal. There was low number of megakaryocytes, surrounded by few platelets.

The patient was BCG vaccinated at birth, but repeated tuberculin tests (1 mg) were negative. After polio vaccination no antibody formation as seen. No coli antibodies ere demonstrated at the age of 3 years. Antistreptolysin titer was 400 and antistaphylolysin titer 2–4 unit Blood group O Rh(+). No isoagglutinins ere demonstrated at the age of 1 and 2 years. At 3 years of age he had titer of /ₘ against A_1 cells, against A_2 cells and against B cells, but during the next year it diminished to titer of / against A_1 and A_2 cells and against B cells. No platelet agglutinins ere discovered N complement fixing antibodies ere demonstrated to Candida albicans, Histoplasmosis, Herpes simplex or Cytomegalovirus



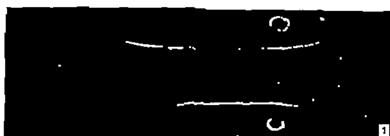


Fig 1 Immunoelectrophoretic analysis. Electrophoretic separation of serum from Case 1 (upper circular basin) and normal serum (lower circular basin) developed with polyvalent immunoglobulin antiserum in upper photograph and with immunoglobulin A antiserum in lower photograph.

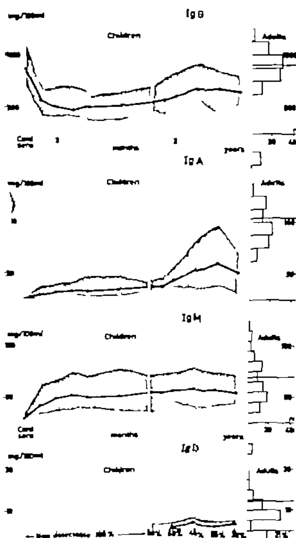


Fig 2 Normal values of immunoglobulins in children. (S. G. O. Johansson and T. Berg, *Acta Paediat Scand.* 56 572, 1967.)

Biopsy of lymph node at the age of 7 months showed hypertrofic reticel cells with infiltrates of eosinophilic cells and more sparsely lymphocytes and plasma cells. The thymus was of normal macroscopic appearance at the post mortem examination.

Immunoelectrophoretic analyses and immunoglobulin determination demonstrated high level of IgG, IgA and IgND (Fig 1, Table 3).

Case 2 M. Je., born on March 2, 1964. Younger brother to case 1. Because of the disease of the brother this patient was examined immediately after delivery. He seemed to be in good health, but the number of platelets was 30,000/mm³. The second day of life slight melena started and continued for 4 weeks. At the same time he developed petechiae, which have persisted since. Because of the pronounced milk allergy of his brother the patient was never given cow's milk during the first year of life, he was fed with breast milk and later with formula of soya beans. From the age of 4 months he has had repeated upper respiratory infections and furunculosis. Staphylococcus aureus and haemolytic streptococci have been cultured from the throat and the skin.

During the *following* years the patient *suffered from* the repeated bleeding manifestations, epistaxis, gastrointestinal bleedings, haematomas and petechiae, which required many transfusions. His susceptibility for infections has decreased during the last year but during this year he has developed slight eczematoid changes. He has developed moderate lymphadenopathy but no hepatosplenomegaly.

Laboratory findings were. Haemoglobin 4.5–8.8 g/100 ml. Reticulocytes 0.4–2.7%. Leucocyte count 4000–8000 with normal differential count, except for eosinophilia.

Table 1 *Clinical and laboratory findings*

Case	1	2	3	4	5	6
Eczema	+ + +	(+)	+	+ +	+ −	− +
Onset	2 months	1 year	2 months	2 months	1 month	1 month
Bleeding manifestation	+	+ + +	+ +	+	+	+ −
Melena in the newborn period	+	+	−	−	−	
Onset of other bleeding manifestations	2 years	2 months	2 months	days	2 months	years
Diarrhea	−	+ +	−	+	+	−
Upper resp. infections	+ + +	+ +	+	+ +	+	−
Otitis	+ +	−	−	+	−	+
Skin infections	+ +	−	(+)	(+)	−	− +
Arthralgia with fever	+	−	−	−	−	−
Platelets	20,000–60,000	15 000–150,000	14,000–160,000	21,000–160,000	20,000–240,000	8,000–60,000
Correlation between bleeding manifestations and platelets	−	−	−	−	−	(+)
Total eosinophil count	2000–4000	300–1600	Normal	300–1500	300–1000	1400–3000
Micro sedimentation rate, mm/1 hour	40–60	54–77	Normal-45	Normal-30	Normal-30	48–80

Total eosinophil count 300–1200/mm³ Micro sedimentation rate 54–57 mm. Serum iron 40–80 µg/100 ml. Platelets 5000/mm³ Bleeding time 8–60 min. Clotting factors normal. Prothrombin consumption pathological. Clot retraction poor No fibrinolytic activity Bone marrow examination revealed an increased, toxic influenced myelopoiesis, reduced number of lymphocytes, normal number of plasma cells, rather few megakaryocytes with reduced number of platelets around.

The patient was BCG vaccinated at birth but tuberculin test (1 mg) was negative. Antistreptolysin titer was 200–400 and antistaphylolysin titer 2 units. Blood group B Rh(+). Isoagglutinins have increased from titer of / against A_1 and / against A_2 cells at the age of one year to $/_{32}$ against A_1 and $/_{16}$ against A_2 cells one year later No platelet agglutinins were discovered. Roentgenological examination has not revealed any lymphoid tissue in the epipharynx. The palatine tonsils were normal in size. Immunoglobulin determination demonstrated high levels of IgA and IgND (Table 3).

Treatment The patient was fed without cow' milk during his first year of life, then he had free diet. His

Table 2. *Immunological status*

Case		Isoagglutinins A	A_2	B	Antistreptolysin titer	Antistaphylolysin titer	Pathological lymphnodes	Tuberculino	Adenoid	Tonsils	Thymus
1	2 years	8	0	0				Neg.			Normal Macroscopic postmortem
	3 years	32	16	2							
	4 years	2	2	4	400	2–4	+	Neg	0	Normal	
2	1 year	8	32	—							N[illegible]sible on x-ray
	2 years	2	16	—	200–400	2	+	Neg.	0	Normal	
3	1 year	8	2	8				Neg.			Not visible on x-ray
	1½ years	64	—	256				Neg.	0	Normal	
4	10 months	—	—	8			+	Neg	0	Normal	Visible on x-ray Normal in size
5	3 months	—	—	0				Pos.			Not visible on x-ray
	6 months	—	—	4	< 100	0 6	+	Neg	0	Normal	
6		Not tested			200–400	4		Neg		Normal	Normal macroscopic postmortem

Table 3 *Levels of immunoglobulins in mg per 100 ml in cases 1–5*

Case	Age (years)	IgA	IgD	IgG	IgM	IgND[a]
1	4 /	230	< 1	1230	91	5 10^{-6}
	4 /₁₂	1060	< 1	1850	81	610 10^{-6}
	5 /₁₂	1190	< 1	1230	30	150 10^{-6}
2	1 /₁₂	230	< 1	930	90	7 10^{-6}
	2	330	15.3	1230	30	78 10^{-6}
3	1 /₁₂	140	14.5	1680	76	73 10^{-6}
	1 /₁	160	[b]	1300	38	[b]
	1 ¹⁰/₁₂	130	7 8	760	48	[b]
	2 /₁₂	280	6.3	1630	43	[b]
4	/₁₂	50	< 1	750	22	13 10^{-6}
	/₁₂	160	< 1	1350	60	[b]
	¹⁰/	210	< 1	1 40	68	[b]
5	/₁₂	88	2.8	730	36	[b]
	/₁₂	180	3.9	935	42	[b]
Mother of cases 3–5	20	140	[b]	1640	220	5 10^{-6}

[a] Level in normal adults: mean, 33 10^{-6} mg/100 ml; range, $11 \times 10^{-6} - 140$ 10^{-6} mg/100 ml (from S G O Johansson, H Bennich and L. Wide, 1968).
[b] Not analyzed.

infections have been treated with antibiotics. Once in a while steroid ointment has been used on his eczema. His bleeding tendency has been the main problem. Except for local treatment of epistaxis and haematomas he has needed blood transfusion every second month. The bleeding tendency has decreased slightly for a few days after each transfusion. Transfusion of fresh plasma, 15 l/kg body weight gave no changes in the number of ..atelets, but the bleeding manifestations diminished. He ... not ...equire any blood transfusion during the following 8 months

Case 3 O J born on May 5 1964 First son of healthy mother A non identical twin brother is healthy No known consanguinity in the family Two younger brothers have the same disease (Cases 4 and 5) but otherwise no member of the family has had similar disorder Purpura and eczema was first noted at the age of two months. After that he was admitted to the hospital in his home area at several occasions because of epistaxis and eczema He has not had any gross intestinal haemorrhages, no otitis media or severe infections. Once he has had sinuitis and twice gluteal abscesses. He has had several upper respiratory infections.

H has twice been admitted to the Department of Pediatrics, University of Umeå, in May 1965 and in January 1966. Physical examination revealed moderate eczematoid lesions at various parts of the body and moderate lymphadenopathy Petechiae have constantly been found, especially in the face As rule he has presented several echymoses and minor haematomas.

Laboratory findings were: Haemoglobin 7 7 11.2 g/100 ml. Reticulocytes 0.1 5..% Leucocyte count 4400–10 400 with normal distribution of the cells. Total eosinophil count within normal limits. Micro sedimentation rate normal to 45 mm. Serum iron 30–68 μg/100 ml. Platelets 14,000–160,000. Bleeding time prolonged. Clotting factors normal. Prothrombin consumption test pathological. No clot retraction. Fibrinogen and fibrinolytic activity slightly elevated (stress reaction?). Bone marrow examination revealed increased erythropoiesis, increased myelopoesis, normal number of lymphocytes, reduced number of plasma cells, normal number of megakaryocytes which were larger than normal, but with few platelets around.

The patient was BCG vaccinated at birth, but tuberculin test (*1 mg*) was negative. Passive cutaneous anaphylaxis demonstrated antibodies to cow milk. Blood group O Rh(+). Isoagglutinin titer at 13 months of age was / against A_1 and B cells and at 20 months / and / respectively No platelet agglutinins were discovered The palatine tonsils were normal in size. Thymus has *never been visible at roentgenological survey nor the* adenoid in the epipharynx. Immunoglobulin determinations demonstrated high levels of IgA and IgND (Table 3).

Treatment Symptomatic treatment of the eczema including local application of steroid ointments has been used Elimination diet has not proved to be of any value. Antibiotics have been given against infections ..th good effect. Gammaglobulin or adrenal corticoid steroids other than locally have not been given. The disease has so far tended to run a mild course with rather mild infections, several episodes of epistaxis but no life-threatening bleedings and a moderate eczema.

Case 4 R J born on May 29 1965 Brother to cases 3 and 5 Petechiae were found at the age of 2 days. He was admitted t the Department of Pediatrics, University of Umeå in June 1965. He stayed there for about months. He had constantly petechiae during this time. Eczema appeared at the age of two months. He had no infections except subclinical urinary infection (coli) *treated with sulfonamides. After this period he has been* admitted to hospital several times in his home area. He has had otitis media several times and bloody diarrhea once. He has also suffered several minor upper respiratory infections. His eczematoid lesions have as rule been very pronounced, sometimes with secretion. He was admitted t Umeå the second time in January 1966. Physical examination revealed pronounced eczematoid lesions, partially secernating, over parts of the body especially in the face and on the trunc. There was moderate lymphadenopathy Petechiae were constantly found. He often had several echymoses and minor haematomas.

Laboratory findings were: Haemoglobin 9.2–11.9 g/100 ml. Reticulocytes 0.5–3.5% Leucocyte count 3500–14,200 with normal distribution of cells, except for eosinophilia. Total eosinophil count normal to 1550/mm³ Micro sedimentation normal to 30 mm Serum iron 130 μg/100 ml. Platelets 21,000–160,000. Bleeding time normal to more than 30 minutes. Coagulation time normal. Prothrombin consumption pathological. Clot retraction poor. Bone marrow examination revealed normal or slightly increased erythropoesis, increased myelopoesis, eosinophilia, increased number of lymphocytes Very few

plasma cells are seen. Megakaryocytes occurred in normal amounts but the platelets were very few around them. He was BCG vaccinated at birth, but tuberculin test (1 mg) was negative. Blood group A Rh(+). Isoagglutinins at 10 months of age were absent. N platelet agglutinins were discovered. The palatine tonsils were normal in size. Thymus was still visible and normal in size at roentgenological survey in March 1966. No lymphoid tissue could be observed in the epipharynx. Immunoglobulin determination demonstrated high levels of IgA and IgND (Table 3).

Treatment. Symptomatic treatment of the eczema including steroid ointments has been used with good result. Elimination diet has not been of any value. Antibiotics has been given against infections and seem to have been of value. Gammaglobulin and adrenal corticoid steroids other than for local application have not been used. The disease has tended to run rather mild course so far although the patient has had several episodes of otitis media and rather severe eczema. The bleeding manifestations have mainly been localized to the skin.

Case 5. S. J. born on Aug. 17 1966. Brother to cases 3 and 4. He had no symptoms during the neonatal period but platelets were 50–60,000, and bleeding time 6 1/2–12 minutes. Eczema was *noted at the age of 1 month and* petechiae at months. He was admitted to the Department of Pediatrics, University of Umeå in October 1966. He had moderate eczema and petechiae. H also had an upper respiratory infection with dense maxillary sinuses. After the discharge he spent most of his time in hospital in his home area because of repeated diarrhea, once with haematemesis and melena, severe eczema and slight upper respiratory infections. He was admitted to Umeå the second time in February 1967 because of rapidly falling levels of platelets. Physical examination revealed at this time rather few eczematoid lesions, but petechiae over most of the body. There was moderate lymphadenopathy.

Laboratory findings were: Haemoglobin 9.8–11.9 g/100 ml. Reticulocytes 1.6–3.4%. Leucocyte count 3900–10,900 with normal distribution of cells, except for eosinophilia. Total eosinophil count from normal to 1034/mm³. Micro sedimentation rate normal to 30 mm. Platelets 20,000–210,000. Bleeding time normal to 9 minutes. Coagulation time normal. Prothrombin consumption pathological. Clot retraction normal. Bone marrow examinations revealed normal erythroid series, increased myeloid series, eosinophilia, rather few lymphocytes and no plasma cells. Rather few megakaryocytes could be seen ith very few platelets around.

The patient as BCG vaccinated at birth. Skin tuberculin test was positive at the age of 3 months. Three months later both skin test and intracutaneous test (1 mg) are negative. Blood group A Rh(+). Isoagglutinins at 3 months were absent, at 6 months / against B cells. No platelet agglutinins were demonstrated. Antistreptolysin titer was under 100, and antistaphylolysin 0.6 units. The palatine tonsils are normal in size. Thymus was not visible at roentgenological survey in October 1966, nor the adenoid in the epipharynx. Immunoglobulin determination demonstrated high IgA and IgND levels for his age (Table 3).

Table 4 *Results of immunoelectrophoretic analysis by different authors*

Quantitative estimation of different fractions are made in the cases of West *et al.*, Ungari *et al.*, Wolff, and in the present study

	No. of cases	IgG	IgA	IgM
Kildeberg (1961)	1	High	High	High
West *et al.* (1962)	2	Normal	High	Normal
Palmgren *et al.* (1963)	1	Normal	Normal	Low
Root *et al.* (1963)	1	Normal	Normal	Normal
Kastrup (1965)	1	Low	Normal	Absent
Dullot *et al.* (1965)	1	Normal paraprotein	Low	High
Lamkowsky (1965)	1	Normal	High	Normal
Ungari *et al.* (1966)	1	Normal	Normal	Low
Wolff (1967)	5	Low-high	Normal-high	Low
Present study	5	Normal-high	High	Normal

Treatment. Symptomatic treatment of the eczema including steroid ointments has been used with good results. Elimination diet has not been of any value. Antibiotics have been given against infections and seem to have been of value. Gammaglobulin and adrenal corticoids other than for local application have not been used. The disease has tended to run relatively mild course so far with mild infections, rather severe eczema and bleeding manifestations mainly localized to the skin.

Case 6. U. A., born on Jan. 14, 1955. First son of healthy mother. No consanguinity as known in the family, no similar disease or any case of death in childhood in the family.

At the age of one month the patient developed an eczema hich increased to generalized severe eczema during the following months. Some improvement was obtained with local treatment. At the age of 2 years he suddenly got susceptible to infections. He had almost continuously upper respiratory infections, skin infections and otitis media. A few months later he developed bleeding manifestations with petechiae, haematomas and epistaxis. He was admitted to the Pediatric Department, Karolinska sjukhuset, Stockholm. At admission he was pale and had eczematoid changes with bleedings all over the body partly infected. Besides he had haematomas of different sizes. He was diagnosed as an idiopathic thrombocytopenia with secondary anemia. He was treated with

prednisolone ~ mg/kg body weight during 4 weeks without effect on the platelets or the prolonged bleeding time. During the next year he still had thrombocytopenia and the treatment with prednisolone was repeated twice without any effect. Because of the invalidizing bleeding tendency splenectomy was performed at the age f 3 years. Immediately afterwards the number of platelets was normalized as was the bleeding time. However the first year after operation he had many overwhelming infections, sepsis, pneumococcal meningitis and severe pneumonias, but after a year his susceptibility to severe infections as well as to upper respiratory infections disappeared and he was in a fair condition with moderate eczematoid lesions. Once he got high fever arthralgia, swelling of both knees and an elevation of the sedimentation rate t 105 mm. He recovered without treatment in 6 days. At the age of 6 years he suddenly got high fever headache, petechiae and went into chock, four hours later he expired.

Laboratory findings were: Before splenectomy: Haemoglobin 5 6–9 g/100 ml. Reticulocytes 0.2 1.6%. Leucocyte count 6300–19,600 with maximum 19% eosinophils. Micro sedimentation rate 48–80 mm. Platelets 8000–60,000. Bleeding time over 30 minutes. Clotting factors normal. Clot retraction poor. Prothrombin consumption pathological.

Laboratory findings after splenectomy: Haemoglobin 7.6–9.8 g/100 ml. Reticulocytes 0.5–2.1%. Leucocyte count 7800–21,000 with 11% eosinophilic cells. Platelets 180,000–260,000 except for the last admission, when they had dropped t 42,000. Bleeding time normal. Clotting factors normal. Clot retraction somewhat poor. Prothrombin consumption border value. Bone marrow at 1 year of age completely normal. Bone marrow examination *at 3 years of age, before splenectomy demonstrated* reduced number of megakaryocytes and an increased number of plasma cells and eosinophilia.

H was BCG vaccinated at birth, but tuberculin test (1 mg) was negative. Isoagglutinins were not tested. Antistreptolysin titer was 200–800 and antistaphylolysin titer maximum 4 units.

The post mortem examination showed suprarenal bleedings, which filled the medulla with blood clots. The other organs did not demonstrate any changes macroscopically except some petechiae in the mucous membranes and the pericardium. The lymphnodes were generally somewhat enlarged. The thymus had weight of 77 g and it did not show any macroscopical changes.

Microscopic examination of the spleen demonstrated hyperplasia of the red pulp, enlarged sinusoids and numerous but rather small Malphigi follicles, which showed eosinophilia.

DISCUSSION

All six patients presented here show the main symptoms of Aldrich syndrome susceptibility to infections, eczema and bleeding tendency and as could be seen from the case reports, any of these symptoms can dominate the disease while the others can be slight or severe.

Increased tendency to infections has occurred in all our patients, 2 of them have suffered severely. A diminished resistance to bacterial infections is a constant finding in earlier described cases. The bacterias causing infections in our *patients are of the commonly occurring types.* The lack of antibodies to *E. Coli* (Case 1) favour the theory of poor formation of circulating antibodies to bacterias. However the antistreptolysin titer was around 400 units in 3 of 4 investigated cases. The increased susceptibility to severe bacterial infections after splenectomy was marked in our Case 6, as it seems to have been in other described cases. All described splenectomized patients died within one year after splenectomy and 8 of the 11 deaths were due to overwhelming infections (8 9 11 12). This reaction seems to be similar to that in infancy after splenectomy which has been explained by the production of IgM of the spleen during early development and *the importance of IgM for the immune reactions* during that age (7). The marked resistance to infections in our Case 6 after the first year post splenectomy was striking and is difficult to explain from the immunological point of view

Our patients have not had any of the common viral children's diseases as morbilli, varicellae and parotitis.

With the exception of three cases with fulminating herpes simplex infections (10) patients with Aldrich syndrome do not seem to suffer more from viral diseases than other children (24). In spite of this patients with Aldrich syndrome seem to have a poor antibody response after morbilli and parotitis (24), as well as after vaccination against viral diseases *(19 24), (Case 1* reported here) as if there might be a defect in the production of circulating viral antibodies, but the cellular response could be intact. The constant finding in our cases of negative tuberculine reactions after BCG vaccination is then puzzling. However no cases of tuberculosis in patients with Aldrich syndrome have been reported.

The low levels of isoagglutinins in the sera could also be due to a change in the formation of circulating antibodies. Low levels of isoagglutinins is a constant finding in these patients, first described by Krivit & Good (19). The remarkably high levels in our Case 3 are surprising and unexplained.

Concerning organs involved in the immune

system we found normal palatine tonsils in all our patients. No lymphoid tissue in the epipharynx could be seen on roentgenological examination in 5 investigated cases. In 3 cases we saw a normal-sized thymus. In the other cases, all still alive, thymus was not visible on roentgenological examination.

The tendency to severe infections and the poor antibody response observed in many cases of Aldrich syndrome would suggest pronounced changes in the immunoglobulins as in hypo- or agammaglobulinemia. Quantitative determination of the immunoglobulins in 5 of our cases showed a high IgA level in all. The presence of elevated IgA level in our cases is in accord with the findings of West *et al.* (30). All our patients had rather high IgG levels for their ages. In case 5 it was possible to show an IgD level of 3.9 mg% although the patient was only 2 months old. Johansson & Berg (16) found IgD in only one of 70 healthy infants below one year of age.

The existence of a new class of human immunoglobulins, called IgND was reported by Johansson *et al.* 1968 (14). This immunoglobulin class has many properties in common with skin sensitizing antibodies, reagins (13 2) and it was later shown (28) that IgND could block passive sensitization in human (Prausnitz-Küstner reaction). Quantitative determinations of IgND in serum from healthy individuals and patients with allergic diseases such as asthma and hay fever (15) showed that elevated concentration of IgND could be found in serum from patients with allergic diseases. The mean IgND level in healthy individuals was 33×10^{-3} mg per 100 ml (range $11-140 \times 10^{-3}$ mg per 100 ml) compared to a mean level of 120×10^{-3} mg per 100 ml (range $13-590 \times 10^{-3}$ mg per 100 ml) in patients with proven allergy

It is therefore of interest to find elevated concentrations of IgND in samples from three (Cases 1 and 3) of the four patients. In Case 4 only a sample from months of age was available for analyses, and found to be normal compared to adults. However since IgND does not seem to pass the placenta (15) even this value might be high for the age.

There are remarkable variations in the immunoglobulin levels from time to time in our patients, which cannot be explained by the increasing age. This is especially true for the IgND levels in Case 1 and Case 2 but also for the IgA levels in Case 1 (Table 3).

The results of immunoelectrophoretic analysis in other described cases, as well as in ours, are summarized in Table 4 (5 17 18 21 24 7 29 30, 32). There are wide variations in the results. This might illustrate the real situation, but rather few of the investigations were made with quantitative methods, which are more reliable.

It has not been possible to classify Aldrich syndrome among any other group of immunological diseases. Many of the symptoms of these patients are also found in patients with agammaglobulinemia. This might be due to alterations of the gammaglobulin molecules, which impose on their function as antibodies, though immunoelectrophoretically no pathological gammaglobulin could be demonstrated in most cases. However Dalloz *et al.* (5) describes one case, which they claim had a paraprotein.

The eczema might be another aspect of the changed immune response in these patients. In some cases (Case 1 and) milk free diet seemed to improve the eczema, though no changes were obtained in the number of platelets or in the susceptibility to infections with milk free diet. Other kind of food can also provoke allergic responses as bloody stools and swelling of the joints (4). A probable allergic response with arthralgia and fever was obtained by oxacillin and probably also following an injection of gammaglobulin in Case 1 and of unknown cause in Case 6. Noteworthy is the increase in the eosinophil count, which seems to be more marked than in patients with common eczema.

Our 6 patients showed thrombocytopenia but only 2 of them had disabling bleedings. The splenectomy in one of them normalized the platelet count and the patient had no bleeding manifestations for 3 years. Just previous to his death a sudden drop in the platelets occurred and he expired in an adrenal haemorrhage. This cause of death has only been described in 3 patients and all of them were previously splenectomized. As well in our 2 patients with severe bleeding manifestations as in the others no certain correlation between the numbers of platelets and the bleeding manifestations was observed. Trials to correct the number of platelets with transfusion of fresh plasma were not successful. This finding is in accord with that of Krivit *et al.* (20) who

also found normal survival time of transfused platelets as did Pearson *et al.* (25) But, though the number of platelets remained unchanged the bleeding manifestations diminished in Case 2. This might suggest not only a decrease in the number of platelets or a defect of the platelets but also some other abnormality in the haemostasis, though no other defect in the clotting mechanism has been proved.

The cause of the thrombocytopenia is obscure. Its presence already at birth is contradictory to the possibility of an auto-antibody induced thrombocytopenia, as is also the lack of agglutinins against platelets. A defect of the megakaryocytes might be expressed by their diminished ability to produce platelets as viewed by the fact that the normal amount of platelets around the mega karyocytes seems to be diminished. However other immunological disorders also demonstrate changes in the number of platelets, e.g. increased numbers are frequently seen in subsepsis allergica and in rheumatoid arthritis.

The presence of immunological deficiencies in these cases of Aldrich syndrome is obvious. At present, however it is not possible to place Aldrich syndrome in the scheme of development of immunological deficiency diseases, as it is presented by Peterson *et al.* (26) and others.

SUMMARY

cases with Aldrich syndrome are presented, one of them splenectomized at the age of 3 died at the age of 6 years of adrenal haemorrhage. Another patient died at the age of 6 years of pneumonia with empyema and meningitis. The other 4 patients ($^1/_2$–6 years) are alive in a fairly good condition. Bleeding tendency dominates the symptoms in 2 cases, in the other 2, susceptibility to infections and/or eczema are the main symptoms. The bleeding tendency does not seem to be correlated to the number of platelets.

Immunological studies in 5 of the patients have revealed a normal or high content of IgG in 4 of 5 cases and an increase of IgA in all. In 3 of 4 investigated patients elevated concentrations were demonstrated of a new immunoglobulin class, IgND The immune response is changed, antibody production by vaccinations against viral diseases was poor tuberculin reactions after BCG vaccination were negative, the pharyngeal lymphoid tissue was absent, and in 4 of the 5 investigated cases low titers of isoagglutinins were demonstrated.

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THE EFFECTS OF ENVIRONMENTAL TEMPERATURE CHANGES ON THE METABOLIC RATE OF NEWBORN BABIES

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Although there are published records of metabolic rates in babies from as long ago as the end of the last century (7 8) it is only in the last decade that metabolic rates have been accurately related to the thermal environment. A neutral thermal environment is that in which the oxygen consumption of the baby is minimal. The theoretical reasons for accepting such a concept were outlined by Hill (4), but she provided no evidence that metabolic rates do in fact increase at high environmental temperatures, and recent work has shown that under suitable conditions environments up to at least 40°C do not cause an increase in metabolic rate (1). In the course of many observations of newborn babies at the Hammersmith Hospital it was noted that a baby's consumption was not only related to the ambient temperature but also to whether the preceding ambient temperature had been higher or lower. Thus babies brought from a room temperature of 20–23 C to 30°C behaved as if comfortably warm being quietly asleep, whereas when cooled from 40°C to the same temperature they almost always appeared to be uncomfortably cold, being awake and restless. The likely explanation for this phenomenon seemed that in the babies whose environment had been cooled to 30 C the skin-environmental temperature gradient was greater than in those whose surroundings had been warmed up to 30°C (1). The following experiments were carried out to determine if this was the sole explanation.

MATERIAL AND METHODS

Rates of oxygen consumption were measured in closed circuit apparatus described previously (10) and being modification of an apparatus described by Hill & Rahimtulla (5). Since the measurements made reflect volume changes within the circuit, leaks were carefully sought before each baby was tested and any present traced and eliminated. Changing the temperature of the air in the circuit causes a temporary change in volume and therefore the time needed to reach a new stable volume was allowed for by carrying out dummy runs at varying temperatures and using changes similar in range and rate to those actually employed during the study of the babies. An increase of 5°C required 12 minutes and a decrease of 5°C 8 minutes for the volume to reach a new equilibrium, which then remained stable. Measured aliquots of air were repeatedly withdrawn from the closed circuit by syringe at various temperatures to ensure that calibration was accurate at these temperatures.

The subjects were 27 normal full term babies from the postnatal wards. Gestational ages ranged from 38–41 / weeks, birth weights from 2700 to 3660 g and at the time of study were 3–7 days old (Table 1). Each infant was studied only once. Permission for each study was obtained from the baby's mother who was invited to observe the proceedings. All babies were fed by breast or bottle not more than 30 minutes before the study but the quantities taken were not determined. (The specific dynamic action due to feed would be expected to have more effect in the early part of the study and then, since babies were studied in alternating sequences, does not affect the general purport of this paper)

The babies were placed naked on 1 5 mm plastic stretcher lined with disposable napkin and kept in the metabolic chamber for at least 20 minutes before measurements began. Temperature measurements using thermocouples were recorded from the following sites: colon (inserted 10–12 cm from the anus), interscapular skin, abdominal skin, post-sacral skin, pre-tibial skin, external water bath, room air, and air entering the chamber (dry and wet bulb) these temperatures were written out automatically on multipoint recorder

Once the infant was placed in the chamber he was left undisturbed throughout the period of observation. A record was kept of the baby's activity at 1 2 minute intervals with the scoring system used by Scopes (10) (Table 2) Only babies showing activity 0 at 35°C were included in the study though the reactions of other babies were observed for clinical correlation. Infants showing activity

Table 1. *Gestation, birth weight and age of babies studied*

1. *Gestational ages, in weeks completed*

Weeks	38	39	40	41
No. of babies	5	6	8	7

2. *Birth weight, in grams*

g	2700	2800	2900	3000	3100	3200	3300	3400	3500	3600
No. of babies	3	2	4	3	5	4	3	2	1	2

3. *Age, in days*

Days	3	4	5	6	7
No. of babies	2	7	13	2	3

1-2 at 30°C or 40°C were left undisturbed and provided they had been at one or the other temperature for at least 15 minutes ere returned to an ambient temperature of 35°C. If they then settled down to activity 0 within 5 minutes the measurements at this temperature were obtained in the usual way and included in the analysis. All others were excluded from the study. For these reasons there are more measurements available for analysis at 35°C than at 30°C or 40°C. The babies were divided into groups. One of these (group W) was placed in the chamber at an ambient temperature of 30°C warmed to 35°C then to 40°C and finally cooled to 35°C. (For the purposes of this paper ↑35 is used to denote an environmental temperature of approximately 35°C achieved by warming the environment from previously cooler temperature. Similarly ↓35 denotes an environment of approximately 35°C achieved by cooling.) The other group of babies (group C) was cooled from an initial 40°C to 35°C then 30°C followed by warming to 35°C again (see Fig. 1). Initially alternate babies were included in the two groups but eventually more babies were studied in group W than in group C. This was because babies tended to wake up as the environmental temperature fell and because statistical analysis demonstrated no significant differences in the metabolic rates of the two groups (In other words the order in which cooling or heating to 35°C was carried out appeared immaterial.)

The temperatures 30°C, 35°C and 40°C were chosen because what is thought to be the "neutral range" lies between these extremes and because 5°C is a convenient range. At the upper and lower extremes it was, of course, not necessary for the environmental temperatures to be precisely 30° and 40°C and in fact the mean temperatures were 30.1 (±0.5°C) and 39.8°C (±0.5°). At the intermediate temperature (35°C) special control was necessary when this was reached for the second time (i.e. position

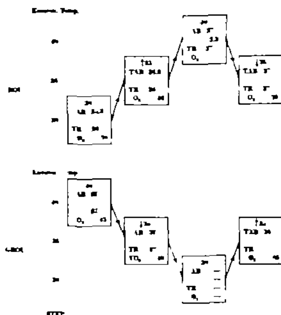


Fig. 1. The sequence of studies that were made on babies in Group W and in Group C showing the environmental temperatures (and the symbols used in the text to describe the conditions obtaining) together with body temperatures and rates of oxygen consumption at each step. The time taken to proceed from Step 1 to Step 4 was 2 2/ hours. *TAB* Mean abdominal skin temperature °C, *D* mean skin-environmental temperature difference °C, *TR*, mean colonic temperature °C, *VO* mean oxygen consumption in ml/kg min.

Table 2. *Activity scoring system employed*

Score	
0	Asleep eyes closed, physically quiet
1	Eyes open but physically quiet or eyes closed with occasional gentle movement
2	Awake with minor irregular and discontinuous movements
3	More continuous and powerful movements
4	Mild crying with considerable activity
5	Continuous powerful movements and lusty crying

4 Fig. 1) On this occasion it was arranged that the temperature gradient between the abdominal skin and air should be the same or slightly greater at ↑35 than at ↓35 in order to eliminate the possibility that the change in oxygen consumption could be thus explained. T achieve these relationships air temperature at ↓35 tended to be slightly higher than at ↑35 although both were well within the range accepted as neutral (↑35 = 34.9 ± 0.5 ↓35 = 35 7 ± 0.3).

Following any rise in temperature in the circuit 15–20 minute period was allowed for equilibration of the volume. When the minute volume of oxygen consumption had been stable for 5 minutes and the dry and wet bulb air temperature had settled at a steady level, a 10–20 minute oxygen consumption measurement was obtained. After reduction of the chamber temperature slightly shorter interval of 10–15 minutes was allowed before the above criteria permitted the measurement of oxygen consumption.

At the final temperature of either ↑35 or ↓35 the baby was allowed to remain undisturbed until he awoke spontaneously and the entire period from equilibration until waking was included in the measurement. The duration of time spent by the babies in the chamber was 2 2 / hours. All the studies were done in air at constant vapour pressure (equivalent to a relative humidity of 65% at 30° falling to 50% at 40°).

RESULTS

There was a consistent reciprocal relationship between ambient temperature and metabolic rate. Oxygen consumption decreased by 1 05 ml/kg/min from 30 C and ↑ 35 and by a further 0.3 /kg/min from ↑ 35 and 40°C. Using paired ~erences the second decrease, although small, statistically significant ($p < 0\ 025$)

When ambient temperatures were reduced from 40 to ↓ 35 the oxygen consumption rose by .3%. The mean metabolic rate at ↓ 35 was 115% compared to that at ↑ 35 a finding that is statistically highly significant ($p < 0\ 005$)



Fig 2. Oxygen consumption of 5 normal infants at different ambient temperatures. The arrows on the lines show direction of temperature changes.

Examples of these observed results are shown in Fig. 2.

The mean colonic abdominal skin and ambient air temperatures are shown in Table 3 together with the corresponding mean oxygen consumptions. The various body temperatures measured showed a consistent relationship to the environmental temperature in that rising ambient temperatures resulted in higher body temperatures and vice versa. The only exception to this to some extent was the colonic temperature, since this was the slowest to respond and also to restabilise. As a result it was often still changing in one direction when the ambient conditions had been reversed. Thus in group W it rose 0.4°C with a change from an ambient ↑ 35 to 40°C and finally settled a further 0.3 C higher but only when the air had been cooled to ↓ 35 On the other hand the abdominal skin temperature rose rapidly by 1 C during the same rise from

Table 3 *Temperatures + VO*

A = Ambient (app) VO = Oxygen consumption in ml/kg/min: mean. ± D ↑35 = approximately 35 C reached by heating: mean ± S.D. ↓35 = approximately 35 C reached by cooling: mean ± D. TD = ambient dry bulb temperature; TR = colonic temperature TAB = abdominal skin temperature TAB-D = abdominal skin — environmental temperature difference; Group W = commenced at 30°C Group C = commenced at 40°C.

	Group W					Group C				
A (app)	TD	TR	TAB	TAB-D	VO_2	TD	TR	TAB	TAB-E	VO_2
30 C	30.1 ± 0 39	36.6 ± 0.12	35.5 ± 0 69	5 4 ± 0.64	6.5 ± 0.81					
↑ 35	34.9 ± 0.27	36 7 ± 0.34	36.5 ± 0.38	1 6 ± 0.28	5 46 ± 0.86	35 0 ± 0.24	37 1 ± 0.1	36.8 ± 0.24	1 9 ± 0.32	5.85 ± 0[illegible]
↓ 35	35.7 ± 0.32	37 4 ± 0.33	37 1 ± 0.38	1 4 ± 0.44	6 39 ± 0.92	35.4 ± 0.50	37 3 ± 0.1	37 1 ± 0.37	1 7 ± 0.40	6.40 ± [illegible]
40 C	39.8 ± 0.51	37 1 ± 0.32	37 5 ± 0.32	−2.5 ± 0.34	5 18 ± 0.39	39.2 ± 0.72	37 4 ± 0.15	37 3 ± 0 42	−1.8 ± 0.38	5.45 ± [illegible]

↑35 to 40°C but fell 0.4 C on returning to ↓35 resulting in a net rise between ↑35 and ↓35 of 0.6 C. The net rise in skin and colonic temperatures between ↑35 and ↓35 in group C were smaller (0.3 C and 0.2 C respectively) *because they spent a shorter time at the inter* vening environmental temperature. Despite these differences in body temperature there were no significant differences in oxygen consumption between groups W and C either at ↑35 or at ↓35 although as mentioned above and shown in Table 3 there were considerable differences when the oxygen consumption at ↑35 was compared with that at ↓35 and this was true of both groups (Table 3). Over the periods of measurement there was very little fluctuation in the gradients between skin and air even over periods as long as 45 minutes, particularly in environments of about 35 C.

Some babies had much higher rates of oxygen consumption than others and this was consistent at each ambient temperature the babies with the highest consumptions having rates 70% higher than those with the lowest. This probably explains the lack of correlation found between oxygen consumption and air-skin temperature gradients at a given ambient temperature (e.g. 29.3–30.8 with abdominal skin-air gradients of 4.5–6.5 C). Nonetheless over wider ranges of ambient temperature and therefore of air-skin gradients there was a significant correlation (e.g. 29.3 35.5°C ambient temperature with abdominal skin-air gradient range of 1.0–6.5 C −0.567 $p <$ 0.001).

DISCUSSION

There is now no doubt that the newborn baby is endowed with homeothermic capabilities. Since he has a higher ratio between his surface area and body mass than older subjects, he must produce more heat per kilogram body weight in any given cooling environment if he is to maintain his body temperature.

All continued heat production is the result of oxidative processes in the tissues. the major sources of heat may be divided conveniently into muscular activity on the one hand and oxidation of substrates in other organs such as brown fat, liver and brain on the other. Muscular action is indubitably a potent producer of heat, but the other processes are also very effec assuming an increasing role in situations o l tle motor activity such as sleep. Babies in cold urroundings tend to be awake, display visible motor activity and may be crying. This activity together with the *other processes producing heat results in a high* metabolic rate. Increasing ambient temperatures initially cause gradual cessation of visible muscle movements, and also a gradual reduction of brown fat activity resulting in a fall in oxygen consumption. However even after all observable muscle activity has ceased, the metabolic rate continues to diminish as the environment becomes warmer and at temperatures as high as 40 C the oxygen consumption is lower than it was at 35 (provided the baby remains inactive). Thus the metabolic rate of 40°C may be 30% lower than it was at the temperature where observable muscular activity stopped. It is essential in this respect to differentiate between short periods of observation (up to 1 hour) following a change in the ambient temperature and observations carried out under conditions of thermal stability lasting for 1. hours or more. In these latter circumstances t has been shown that environments causing a rise in abdominal skin temperatures to over 37°C result in an increase in metabolic rate (11). This means that there is an oxygen cost of prolonged exposure to very high ambient temperatures.

The present study was concerned exclusively with the responses during the shorter periods of observation. Under such conditions there was a very obvious constant pattern of behaviour suggesting that the baby had become adapted to the previous temperature. For instance a rise of 5 C from 25°C to 30°C (i.e. from room to initial chamber temperature) resulted n almost all infants becoming quiet and many of them falling asleep. A change from 35 C to 30 C on the other hand caused an ncreased estlessness in many babies and some even awoke and cried. An even more striking phenomenon was shown by a few babies who appeared to be quite comfortable at 40°C but behaved as if they were cold when the temperature was reduced to 35 C.

This clinical behaviour pattern was borne out by measurements of metabolic rates during periods when visible muscular activity was absent. All the babies in this study were normal and able to maintain their body temperature under the

prevalent nursery conditions (napkin and shirt, 2 blankets, room temperature 23–27 C). There was a considerable variation in metabolic rates between individuals when exposed to similar thermal environments, but the relationship of changes in these rates to any particular change in the ambient temperature was qualitatively constant. Whether the baby was warmed or cooled initially had no influence on these metabolic readjustments. Clearly adaptation to a specific environment occurred rapidly provided such an environment was not too uncomfortable. Once this adaptation had taken place any change in the surroundings was associated with a predictable response from the infant, a response which, however may be inappropriate in the long term and therefore not maintained. For example, the mean oxygen consumption at 30 C was almost identical with that at ↓ 35 while at ↓ 35 it was 1 ml/kg/min higher than at ↑ 35 Since the protocol was arranged to eliminate the possible effects of a gradient between skin and air this phenomenon suggests a response to the changes *per se* Statistical analysis shows the difference between the values at ↑ 35 and ↓ 35 to be highly significant in situations where the only difference between the two sets of conditions was the preceding change.

It has been shown by other workers that ygen consumption correlates best with abdom- l wall skin-air temperature gradient (1). A between these variables was also found in the present study and the wider the range of temperatures the closer this correlation became.

Since the metabolic rate at ↓ 35 was greater than at ↑ 35 and at the same time body temperature was also higher the possibility of a Q 10 effect had to be taken into consideration. The result of this effect would be an increase of some 10% in the rate of chemical reactions in the body for each 1 C rise in temperature. In previous studies (1 5) this effect has always been entirely overshadowed by the metabolic responses presumably mediated by the central nervous system the only infants who show the Q 10 effect clearly are those suffering from very severe congenital cerebral anomalies (3 9). In order to explain a rise of oxygen consumption of 15% on a Q 10 basis, one would expect a change in body temperature of at least 1.5 C. The mean change in group W babies body temperature was only 0.6 C and in group C only 0.3 C, and yet both groups had a similar rise in metabolic rate from ↑ 35 to ↓ 35 The anticipated rise expected from such increases of body temperature was 0.4 ml/kg/min which is significantly lower than the observed rise of 0 9 ml/kg/min ($p < 0.01$). Therefore in terms of the magnitude of the change and the inconsistency between the groups, it seems unlikely that this large change in oxygen consumption would be due to a Q 10 effect.

The baby's response to changes in ambient thermal conditions has a practical consequence in incubator design, for it appears to be important to avoid sudden changes of temperature. Such changes are unlikely in conventional incubators (except when they are opened) but this is not true where radiant heaters are employed using an on-off thermostatic system. When the radiator is turned off, the radiant surface temperature falls by 10°C or more, subjecting the child to a sudden cooling of his thermal environment. Though the end result of an on-off action may be a more or less constant absolute temperature, the sudden changes may evoke a metabolic response in the baby increasing his oxygen requirements by as much as 25% Thus, at least until information is available, it would seem advisable not to use a form of heat control which has an on-off action with large swings of temperature.

The concept of a "neutral" thermal environment needs re-evaluation. Silverman *et al.* (11) have found that babies nursed in a stable thermal situation will have a minimal oxygen consumption when their abdominal skin temperature is maintained at 36–37 C. In a heat gaining environment, even if very warm, there is no increase in metabolic rate until the baby's body temperature has exceeded 37 C providing the baby has not become restless. Thus the true nadir of the metabolic rate is difficult to find. In addition, the direction of approach to the "neutral temperature must be stated, since the criteria hitherto used in defining the neutral zone do not take into consideration the dynamic effects of the previous thermal environment. These findings

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Q 10 effect. A principle (ascribed to van't Hoff, 1896) is that there is change in rate of chemical reaction with change in temperature. Empirical determinations for biological systems have shown an increase in reaction velocity of 2 to 3 for each 10°C rise in temperature.

also cast doubts upon the validity of the concept of critical temperature.

To make results from different centres comparable, a standard environment should be established, since the terms "basal or minimal" are ill defined. An ambient temperature of 35–36 C at about 50% relative humidity an abdominal skin-environmental temperature gradient of 1.0–1.5°C having been reached by warming the environment, or a stable environment of this kind for more than 2–3 hours could provide such a standard environment for most babies. They should be quietly asleep and ideally in non eye movement sleep (6). Ideally also the time and protein content of the last feed should be constant.

The human body is well equipped to maintain the *milieu interieur*. Homeothermy is one of the features of homeostasis. Deviations from the normal levels of many factors can cause clinically observable phenomena. Under normal conditions the blood levels of many chemical substances are controlled between fairly narrow limits. Sudden deviations from these limits may produce symptoms, as in hyponatraemia or hypoglycaemia. In illness the body may become adapted to abnormal levels of these substances and may apparently function well. A sudden change from the abnormal but stable level towards the normal range may then precipitate symptoms. This may be seen in hypernatraemia, where a sudden fall of serum sodium from say 170 mEq/l to 150 mEq/l may precipitate fits, or a reduction of the blood glucose level from 600 mg/100 ml to 200 mg/100 ml may cause coma. Gradual adaptation to the new level on the other hand is unlikely to cause symptoms.

This study shows that there is in this respect an analogy between the adaptation to internal and external conditions. It is a matter of common experience that on a frosty night one feels warm on entering an half heated part of a house from outside, whereas the same part of the house feels cold when entered from a warm living room.

SUMMARY

The oxygen consumption of 27 normal babies was measured at an environmental temperature of about 35 C, approaching this temperature either from 30 C or 40°C.

There was a decrease in oxygen sumption at an ambient temperature of 40 mpared with 35°C provided the baby rem. asleep and motionless.

The oxygen consumption at an env mental temperature of 35 C when this had been ched by coming from 40°C was 15 higher than when it had been reached by warming from 30°C This difference could not be explained by differences in skin-air temperature gradients nor by a Q 10 effect.

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A TECHNIQUE FOR THE ENZYMATIC DIAGNOSIS OF GLYCOGEN STORAGE DISEASE ON VERY SMALL TISSUE SPECIMENS

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In many inborn errors of metabolism analyses on biopsy specimens of the liver are of great value or even indispensable for a definite diagnosis. This is true for glycogen storage disease, especially type I, in which the defect of glucose-6-phosphatase is demonstrable only in liver kidney (4) and intestinal mucosa (8). In other forms of glycogen storage disease (type II, III, IV and VI) a diagnosis may be settled by analyses on blood corpuscles (for a review is referred to ref. 9–10). In some cases, however the results from analyses on blood cells are not clear-cut or even definitely misleading (1, 3 5 9 10) Consequently analyses on liver (and/or muscle) are necessary in many cases of glycogen storage disease.

Up to now liver biopsy specimens for such analyses have been obtained with laparotomy or needle biopsy with relatively large-diameter needle. These procedures for liver biopsy are not without risks (mainly bleeding and acidosis), the risks being greater in patients with glycogen storage disease than in normals.

Because of such dangers, in some cases a biopsy is never performed and the diagnosis remains uncertain.

A less hazardous procedure for liver biopsy utilizes a very fine needle (12). Only very small pieces (0.5-2 mg) are obtained, earlier used mainly for cytological diagnostics. Such small pieces of liver tissue have very little been used for the biochemical diagnosis of metabolic disorders. Because of the greater safety of the fine-needle biopsy procedure as compared to other biopsy procedures, it was considered worth while trying to utilize it for such diagnostics, e g. in glycogen storage disease. However presently available methods for the diagnosis of glycogen storage disease are not sensitive enough to be used on 0.5–2 mg pieces of tissue. It was, therefore, tried to work out more sensitive techniques. The results of such attempts are now presented.

MATERIALS AND METHODS

Chemicals

Phosphorylase limit dextrin was prepared according to Hers (6). All other chemicals used were commercial preparations of highest obtainable purity

Preparation of tissues

Small fragments were cut away at −20° from frozen pieces of liver and transferred without weighing to each one of two all-glass Potter-Elvehjem homogenizers, one containing 0.5 ml ice-cold 0.25 *M* sucrose in 0.001 *M* EDTA, pH 7.0 the other 0.5 ml distilled and deionized water. The fragments were homogenized at 0°. The sucrose-EDTA homogenate was used for assay of glucose-6-phosphatase, phosphorylase, amylo-1,6-glucosidase, and protein and the homogenate in water for assay of glycogen, α-glucosidase and protein as described below

Assays

Glucose-6-phosphatase was measured as described earlier (11) using the "ordinary technique" as described in that reference with incubation times 0-0-1 1 2 2 hours for both substrates (glucose-6-phosphate and β-glycerophosphate) Standards containing 0 to 0.05 μmoles of phosphorus were analysed simultaneously For the assay of *phosphorylase* the following micromodification of Hers method (6) was worked out. 0.005 ml of substrate (containing 0.1 *M* glucose-1-phosphate, 2% glycogen, 0.003 *M* adenosine monophosphate and 0.2 *M* NaF pH 6.1) was incubated at 37° with 0.005 ml of homogenate for 10-10-20-20-30-30 min in conical plastic centrifuge tubes, 14 101 mm. The reaction was stopped by the addition of

Normal values

No liver biopsy specimens from strictly normal children were available. Consequently normal values for glycogen and enzymes cannot be presented. As already discussed in an earlier communication (9) an interpretation of enzyme assays in patients, suspected to have glycogen storage disease, can be made without such strictly defined normal values. Therefore, the conclusions drawn from the results shown in Table 2 should be valid, even though a comparison could only be made with the few results, shown in Table 1

The possibility now in sight to use fine-needle biopsy specimens should, however greatly increase the chances, ultimately to be able to estimate the normal range of enzyme activities and glycogen concentration in children.

Application of the methods in practice

To allow an appreciation of the value of the present methods in practice more experience is needed. Such experience, supporting the applicability of the methods, is now gradually being accumulated through analyses on fine-needle biopsy specimens from patients, suspected to have glycogen storage disease.

SUMMARY

The difficulties to obtain an exact diagnosis in cases of glycogen storage disease are mentioned. New diagnostic methods, utilizing blood cells, have not allowed settling a definite diagnosis in all cases. Analyses on liver tissue, consequently are still needed in many cases. However the methods up to now used for obtaining a piece of liver tissue (surgery or large-diameter needle-biopsy) involve dangers to the patient. Because of this, a less risky biopsy procedure, such as can be performed with a fine-needle, was thought preferable.

To be able to utilize very small amounts of liver tissue, as obtainable with a fine-needle, for the diagnosis of glycogen storage disease, methods were worked out, allowing the analysis of glucose-6-phosphatase, phosphorylase, amylo-1 6-glucosidase, α-glucosidase, glycogen and protein on 0.5–2 mg fragments of liver tissue. The methods involved incubation of very small volumes, assay of phosphorus by a newly described, highly sensitive method and of glucose by the hexokinase-glucose-6-phosphate dehydrogenase method with fluorimetric measurement of the NADPH. Protein and glycogen could be assayed in a more conventional way The stability of the enzyme activities and the precision of the methods is discussed.

Results on liver biopsy specimens from seven controls and on biopsy and autopsy specimens from liver in eight cases of four different types of glycogen storage disease are given and compared to the results on these specimens with earlier described methods.

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FATAL GRANULOMATOUS DISEASE

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In the heterogeneous group of children with increased susceptibility of infections it is in most cases possible to demonstrate lack of antibodies, defects in cellular immunity or granulocytopenia. Patients with severe recurrent or chronic infections are, however, found in whom such defects cannot be demonstrated, on the contrary diffuse hypergammaglobulinemia is frequently encountered (9). In 1957 a specific syndrome was delineated by two groups in USA (3, 12): 6 patients were described, all boys, who since infancy had suffered from frequent suppurative infections, in particular lymphadenitis, besides they had eczem-
s lesions around the eyes, nose and mouth,
us stomatitis. The course was in some cases fluctuating with periods of well-being, but infections always recurred, and most patients died within preschool age. Pulmonary infection portended a rapidly fatal outcome; septicemia occurred only terminally. Microscopical examination of affected organs revealed granulomas with plasma cells, lymphocytes, and macrophages containing a characteristic yellow-brown lipid pigment. Culture was often negative; in positive cases, staphylococci were most frequently encountered. Mycobacteria, fungi, or virus could not be demonstrated. The cause of the increased susceptibility to infections was unknown, in particular most patients exhibited a marked diffuse hypergammaglobulinemia.

This syndrome was called Fatal granulomatous disease (FGD). In the following years several new cases were reported in USA (5), establishing that it is a sex-linked recessive hereditary disorder with a fatal outcome before puberty. Antibiotics cannot prevent this, and other therapeutical measures have likewise proved ineffective. In spite of numerous investigations no defects in humoral or cellular immunity have been found, and the phagocytic capacity of the neutrophil granulocytes is intact.

By incubating bacteria with leucocytes from three FGD-patients Holmes *et al.* in 1966 (8) demonstrated that following phagocytosis the bacteria were not destroyed, but survived intracellularly in the granulocytes. Contrary to normal cells, the neutrophils showed no degranulation or vacuolization following phagocytosis. The fatally increased susceptibility to infections of these patients thus appears to be caused by a defect of the neutrophil granulocytes and perhaps other phagocytic cells as well, and it visualizes the importance of these cells whose function from a phylogenetic viewpoint is considerably older than the specific immunological defence mechanisms.

FGD has only been reported *twice in Europe* (10, 13). In this paper two brothers with FGD are presented together with the investigations performed on the function of the neutrophil granulocytes.

CASP REPORTS

P. F. C., RH dep. G, 1039/52. Boy born in 1949 as the oldest of 4 siblings. Pregnancy and delivery without complications. In early infancy he had diarrhoeas, probably due to allergy to cow milk, and was held for several months on human milk. Also later in life he had every stools and periodic diarrhoeas.

When he was 3 months old recurrent infections began: tonsillitis, lymphadenitis of the neck several times requiring incision, bronchitis and pneumonia. Treatment

with antibiotics had variable effect. In addition he suffered from gingivitis and persistent fissures in the corners of the mouth.

3 years old he was hospitalized with fever and diarrhoea, and until death 6 months later he was almost constantly in hospital because of septic fever and anemia. On admission physical examination revealed slight hepatomegaly and slight enlargement of the lymph nodes, but the spleen was not palpable. Erythrocyte sedimentation rate was increased, up to 97 mm/h. Leucocyte counts were 12,000–20,000/µl, with 80% mature neutrophil granulocytes and 11% lymphocytes. Cultures from blood and spinal fluid were negative. Chest X-ray showed calcified tuberculous lesion in the right upper lobe. On further investigation nothing was found to explain the increased susceptibility to infections. Treatment with a variety of antibiotics was ineffective. Gradually obvious symptoms of a subphrenic abscess developed. It was drained; cultures grew *Staphylococcus aureus*, sensitive to all antibiotics examined together with *Staphylococcus albus* and Klebsiella. In spite of intensive treatment with broad spectrum antibiotics, gammaglobulin and blood transfusions, the child succumbed one month after the operation shortly before reaching 4 years of age.

Autopsy showed liver abscess, empyema, fibrinopurulent pericarditis, and bilateral chronic pneumonia. Histological examination of the lungs, liver and lymph nodes revealed granulomas with central necrosis, surrounded by numerous plasma cells and lymphocytes, macrophages, and a few multinucleated giant cells. Dispersed in the organs many macrophages were found whose cytoplasm contained a yellow-brown pigment staining intensely with Sudan Black B.

T. F. C., dep. G, 969/66. Boy born in 1959 the brother of the first patient. Pregnancy and delivery without complications. He received cow's milk from birth and had during the first weeks of life diarrhoeas, exanthema and conjunctivitis, later also violent vomiting. The symptoms vanished when he was given human milk only. He later showed allergy towards a number of food items, as a result of which he on several occasions suffered anaphylactic shock. Throughout life he remained on a restricted diet.

At 8 months of age, recurrent infections began. Otitis media, lymphadenitis of the neck requiring repeated incisions, blepharitis, paronychia, and eczematous lesions around the nose and mouth. During several admissions he was treated with antibiotics, gammaglobulin, and autovaccination, with little effect. He continued treatment as an out-patient for several years.

At the age of seven, after a symptom-free period of 18 months, lymphadenitis of the neck reappeared, and he was again hospitalized. On admission he was found to be anemic. Liver and spleen were not palpable. Of the cultures from the abscesses in the neck, some were negative, others grew *Staphylococcus aureus* resistant to penicillin, but examination of a lymph node biopsy from the neck showed granulomas with central necrosis and fibrosis; plasma cells, lymphocytes, macrophages, and giant cells were found, and there were pigmented lipid-filled macrophages of the same kind as seen in the brother. Since these findings suggested tuberculosis, the patient was treated with para-amino-salicylic acid and isoniazide. However neither fluorescence microscopy nor culture revealed mycobacteria, so antituberculous treatment was discontinued.

A short time later, a subphrenic abscess developed which was drained. Some improvement followed but he still had septic temperature, and shortly after discharge he died, nearly 8 years old.

Family history

The *mother* of the patients suffers from hay fever. During childhood she had almost constantly aphthous lesions in the mouth, they showed periodic aggravation with fever, sore throat, tonsillar enlargement and swelling of the regional lymph nodes. These symptoms vanished at puberty. The *father* of the patients has diabetes which developed at the age of 30 but is otherwise healthy.

In the marriage were four *children*. The histories of the two sons are presented above. The two *daughters* suffer from attacks of aphthous stomatitis, like their mother.

Nothing is known of increased susceptibility to infections in male members of the mother's family.

SPECIAL INVESTIGATIONS

Patient T.F.C. *Circulating antibodies.* When the patient was 10 months old, his serumgamma globulin concentration was 680 mg/100 ml. Immunoelectrophoresis was normal. At the age of 7 years, the concentration had risen to 1400 mg/100 ml. Immunoelectrophoresis now showed increased concentrations of IgG and IgA. The titers of staphylococcal agglutinins and precipitins were markedly elevated. Thus a defect in the circulating antibodies could not be demonstrated.

Cellular immunity. The lymphocyte count in the blood varied from 3000 to 12,000/µl. The Moro tuberculin test was positive on several occasions, but the Mantoux test was negative. An intracutaneous test with 2×10^4 killed *Brucellae abortus* was negative, but when 2×10^7 bacteria were employed the test was positive with an area of induration of 8 mm in diameter. Ability to develop immune reactions of the delayed hypersensitivity type was thus demonstrated in the patient.

Neutrophil granulocytes. (a) Concentration and morphology. The number of neutrophil granulocytes in the blood varied between 10 000 and 25,000/µl. During the last period of hospitalization the number was approximately 20 000/µl, with a slight shift to the left (the ratio band forms/segmented = 1/5 and a few metamyelocytes) and toxic granulation.

(b) Phagocytic capacity. As early as in 1961 the phagocytic capacity of the patient's neutro-

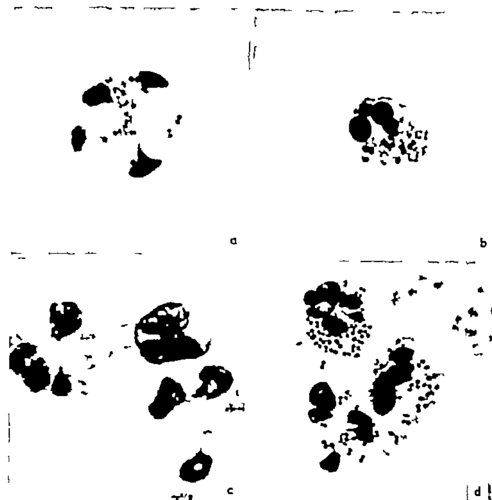


Fig. 1 Neutrophil granulocytes, isolated from heparinized blood by dextran sedimentation and incubated in vitro (37°C). The slides were prepared in a Shandon Cytocentrifuge and stained with May-Grunwald-Giemsa. Magnification × 1200 (a) Neutrophil from Control after 40 minutes incubation with Staphylococcus aureus (isolated from Case 2); ratio staphylococci/neutrophils = 4/1. Pronounced degranulation and vacuolization is seen. (b) Neutrophil from Case 2, incubated as (a). The ingestion of staphylococci has not induced degranulation and vacuolization of the cytoplasm. The nucleus is pycnotic and fragmented. (c) Two neutrophils from Case 2, following 40 minutes incubation in an isotonic medium containing 20 mM/l of sodium fluoride. The cytoplasm is degranulated and vacuolized. (d) Neutrophils from the mother of our two patients, incubated as (a). Two kinds of phagocytizing neutrophils are seen. One granulocyte shows normal degranulation and vacuolization, the two others are without cytoplasmic reaction.

phils was examined, following incubation with heat-killed *Staphylococcus aureus* and found to be normal. During the last period of hospitalization the phagocytic capacity of the neutrophils was reexamined, using *Staphylococcus aureus* isolated from the patient and a medium containing autologous plasma. The relative number of phagocytic cells and the number of bacteria phagocytized per cell did not differ significantly from the results obtained with normal granulocytes.

(c) *Fate of ingested bacteria.* Following phagocytosis of bacteria or inert particles normal neutrophil granulocytes in an oxygenated medium display degranulation and vacuolization as steps in the destruction of invading microorganisms (7) (Fig. 1 *a*). This could not be demonstrated in the granulocytes of the patient (Fig. 1 *b*) neither in autologous nor in homologous serum. The ingested staphylococci remained morphologically intact, and gradually the nucleus of the phagocytizing granulocyte became pycnotic and fragmented, demonstrating the death of the cell.

(d) Induction of degranulation in the patient's granulocytes. Following incubation with klebsiella instead of *Staphylococcus aureus*, degranulation and vacuolization was seen in the patient's neutrophils as well as in normal cells however this was also observed in non-phagocytizing cells. That this effect was due to a toxin, as previously demonstrated with several other bacteria (4), was confirmed by incubating the granulocytes with the bacteria-free filtrate of a klebsiella culture: this also induced degranulation and vacuolization.

In a medium containing sodium fluoride normal granulocytes are degranulated (11). Fig. 1 *c* demonstrates that the patient's granulocytes display degranulation and vacuolization in a medium containing 20 mMol/l of sodium fluoride.

DISCUSSION

The clinical picture of these two brothers was typical of Fatal Granulomatous Disease (FGD), with recurrent infections caused by common pyogenic bacteria, without signs of defects in humoral or cellular immunity. The marked food allergy in both brothers does not, however belong to the syndrome. The histological findings of chronic granulomatous inflammation with tuberculoid structure matches the normal response to intracellular survival of bacteria, e.g. TB as the second brother reported here, several patients suffering from FGD have received antituberculous treatment on grounds of similar biopsy findings (3). Characteristic of FGD were the macrophages filled with yellow-brown lipid; whether this is derived from ingested bacteria is not known. The diagnosis of FGD was confirmed by the demonstration *in vitro* of the defective function of the neutrophil granulocytes (Fig. 1 *b*).

Treatment of patients with FGD has proven ineffective. Leucocyte transfusions have been tried without effect (5), probably due to the short life span of the granulocytes. Our finding that toxic agents can induce degranulation in FGD-granulocytes would not appear to have any therapeutic implications. The only treatment available is antibiotics, notably those that penetrate into cells.

The broad outlines of the mechanism that leads to destruction of bacteria phagocytized by neutrophil granulocytes were clarified with the demonstration that the granules contain catabolic enzymes, thus being lysosomes (6), and that phagocytosis is followed by degranul (7): the catabolic enzymes are released into acuoles containing the ingested bacteria (18) destruction of bacteria accordingly takes pl in the vacuoles, which enlarge during the ocess. Whereas phagocytosis is possible under a robic conditions, since the energy is supplied b gly colysis, the oxygen uptake of the cell mus n crease in order that phagocytized bacteria can destroyed (16): in particular the hexose monophosphate shunt is activated (15).

It is not known how degranulation is induced by phagocytosis, and the defect in FGD is likewise unknown. Baehner & Nathan (1) ha e demonstrated that the lactate production during phagocytosis is increased to the same extent in neutrophils from patients with FGD as in normal neutrophils, however the oxygen consumption of FGD-granulocytes is not increased (17) and the hexose monophosphate shunt is not activated (1). This has been employed in a diagnostic test, in which normal phagocytizing granulocytes reduce nitroblue tetrazolium, whereas FGD-granulocytes do not (1).

The same principle has been used in a histochemical test (17) with this it was shown that female carriers of FGD possess two populations of neutrophil granulocytes, of approximately equal size One that functions normally and one inactive. This is in accordance with Lyon's hypothesis of random inactivation of one of the X-chromosomes in female cells. This defective population probably explains the increased susceptibility to infections, notably stomatitis, of female heterozygotes which was encountered in the family reported here and also in other FGD families (2). Since the number of normal granulocytes of the female heterozygotes should be sufficient for coping with bacteria, the abnormal granulocytes must increase the possibility of survival of invading microorganisms. When the neutrophil granulocytes of the mother of our two patients were incubated with staphylococci, two populations of about equal size were visualized. One which showed normal degranulation and vacuolization after phagocytosis, and one with the non-reactive cytoplasm characteristic of FGD (Fig 1 *d*) in which dividing bacteria could be seen.

Recently an experimental parallel to FGD has been demonstrated (14): The addition of colchi-

cine to a leucocyte suspension does not influence phagocytosis, but suppresses degranulation, vacuolization, and increase in oxygen consumption. The study of such models will possibly elucidate the normal function of neutrophils, and perhaps also the defect in FGD

SUMMARY

Two brothers with Fatal granulomatous disease of childhood (FGD) are presented. Both patients exhibited pronounced food allergy Otherwise, the course of the disease was typical of FGD From infancy recurrent infections, notably suppurative lymphadenitis, and a fatal outcome before puberty

Microscopic examination of affected organs revealed granulomas with tuberculoid structure· the macrophages contained a characteristic yellow brown lipid pigment.

A defective function of the neutrophil granulocytes has been demonstrated in FGD The phagocytic capacity is intact but phagocytized bacteria are not killed since phagocytosis does not induce liberation of catabolic enzymes from the neutrophil granules. In one of our patients, defective degranulation and vacuolization of the cytoplasm of neutrophils was demonstrated *in vitro* following phagocytosis of staphylococci. ... ver exposure to sodium fluoride or kleb- ... toxin did induce degranulation and vacuo- ... of the defective granulocytes.

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EFFECT OF PHENYTOIN ON THE TRYPTOPHAN LOAD TEST

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Three types of abnormal pyridoxine metabolism have been described. In *pyridoxine deficiency*—which may cause fits and retarded growth in early infancy—the tryptophan load test is abnormal and the clinical and laboratory abnormalities disappear when the diet is supplemented with pyridoxine. In *pyridoxine dependency*—an inborn error of the pyridoxine metabolism with fits usually beginning in the neonatal period and responding promptly to large doses of pyridoxine—the tryptophan load test is normal. The characteristic features of these two conditions and the literature in this field have been reviewed by Waldinger (17). In a *third type of pyridoxine-responsive seizures* reported to appear in children of any age the tryptophan load test is abnormal. The clinical response to large doses of pyridoxine is not always as impressive as in the other two groups. The tryptophan metabolism usually becomes normal during treatment (1 ., 4 7 9 12, 14 16).

In a search for the third type of pyridoxine responsive seizures tryptophan load test were performed on children with severe epilepsy admitted to the Department of Pediatrics of Lund. Several children with abnormal tryptophan load tests were found, but their symptoms did not respond to large doses of pyridoxine. It was gradually realized that almost all the abnormal tryptophan load tests were seen in children receiving hydantoin derivatives. The material was therefore extended to include also children with mild epilepsy children with neurological abnormalities without seizures (including some who were mentally retarded) and apparently healthy children. In several cases the tryptophan load test was performed both before and during administration of hydantoin. The only drug used in the final material was phenytoin (Difhydan[1] Leo) The purpose of the investigation was thus to find out whether the result of the tryptophan load test can be influenced by the administration of phenytoin to the children.

MATERIAL AND METHODS

The material includes 27 children (15 boys, 12 girls, aged 3 months–15 years) with epilepsy 10 children (2 boys, 8 girls, aged 4 months–13 years) with various neurological disorders (5 of them were mentally retarded) and 14 children (11 boys, 3 girls, aged 6 months–11 years) who were apparently healthy

The children received phenytoin 3 times a day in a total daily dose of 10 mg/kg body-weight. This medication had been administered for at least 2 weeks before the tryptophan load test during phenytoin treatment was performed, except for 2 of the healthy children who received phenytoin for only 5 and 9 days, respectively No patients were receiving pyridoxine or ACTH when the tests were carried out

The children were allowed no food over night, and they were given 100 mg L-tryptophan/kg body-weight dissolved in milk. Urine was sampled during the following 7 hours and 5 ml 1 *N* HCl was added together with a few millilitres of toluol. The urine collected was kept in a refrigerator until the following day when the amount of xanthurenic acid was determined.

As the results of the tryptophan load test varied with the age of the child, the mean values for the different categories of children were calculated separately for two age-matched groups. Regression equations for the variation of the excretion of xanthurenic acid with the age of the child were obtained by the method of the least squares with the whole material divided into two groups, all children *who did* and all children *who did not* receive phenytoin. The statistical evaluation of the variances within these both groups was performed with Snedecor' F-test. The effect of variation with age was eliminated by analysis of variance. In the evaluation of the results of the tests in patients and control submitted to tryptophan load test both before and during phenytoin administration, allowance for age was not necessary as these sub-

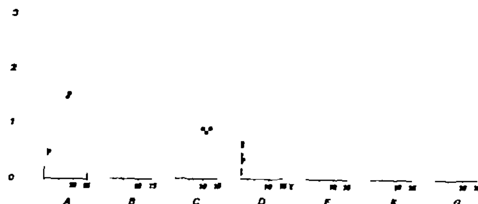


Fig 1 Xanthurenic acid *excretion after tryptophan load in different groups of children; A, Healthy controls; B* healthy controls, receiving phenytoin, *C* neurological disorders, no epilepsy *D* epilepsy before treatment with anticonvulsive drugs; *E*, epilepsy treated with anticonvulsive drugs, but no phenytoin; *F* epilepsy treated with phenytoin only *G* epilepsy treated with phenytoin and at least one more anticonvulsive drug. The ages of the children appear from the age scale at the base of the columns. Each dot represents one examination. As some of the children are represented in more than one column, the number of dots exceeds that of the children tested.

jects served as their own controls, the differences found between the paired observations were treated with the Student's *t*-test with Bessel' correction for small samples.

Measurement of Xanthurenic acid Excretion

urenic acid content of the urine was determined with fluorimetric method mainly according to Satoh & Price (13).

Ion exchange chromatography with column 25×4 mm containing Dowex 50–X8 (100–200 mesh) was performed with acidified and diluted urine. The packed column was rinsed with 3 ml 5 *N* HCl and 5 ml ion-free water.

One ml of the urine sample was diluted with 5 ml ion-free water and 1.5 ml 1 *N* HCl. The mixture was put on the column and chromatographed with rate f 5–6 drops/min. The column was rinsed with 25 ml 2 *N* HCl and 5 ml *N* HCl and eluted with 20 ml ion-free water The eluate was thoroughly mixed after which 3 ml of it was mixed with 3 ml 2 *N* NH_4OH. A blank was prepared with 3 ml ion-free water and 3 ml 2 *N* NH_4OH and xanthurenic acid standard with 3 ml xanthurenic acid (0.5 µg/ml) likewise mixed with 3 ml 2 *N* NH_4OH. Blank, test and standard were read within two hours in spectrophotofluorometer (Aminco-Bowman) under optimal conditions: wave lengths 350/460 nm (uncorrected). The recovery of xanthurenic acid with the described method was 90–100%.

In order to ascertain whether phenytoin in the urine can interfere with the determination of xanthurenic acid, several runs were made on urine to which phenytoin had been added.

RESULTS

The results are summarized in Fig. 1 Each dot represents the result of one tryptophan load test. As several of the children were tested more than once, the number of determinations exceeds that of the children tested. It is apparent, particularly in the children not receiving anticonvulsive drugs, that the excretion of xanthurenic acid following tryptophan loading increases with age (see also the regression lines of Fig. 2).

The mean values for epileptic children taking phenytoin are presented in Table 1 where they are compared with those recorded in age-matched groups of epileptic children not receiving phenytoin and of non-epileptic children. All the determinations are plotted in Fig. 2. The variation of the result of the tryptophan load test with age is illustrated by the regression lines. The regression coefficients are significant (F-test, $p < 0.01$). The variation within the group of all children receiving phenytoin is larger than within the group of children not receiving phenytoin ($p < 0.01$). After the elimination of the effect of variation with age this difference was still significant ($p < 0.01$). The differences between the groups of children not receiving phenytoin (epileptic as well as non-epi-

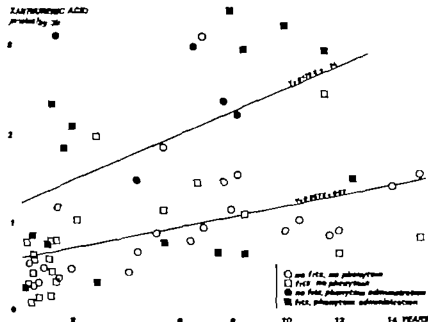


Fig 2 Xanthu... excretion *related* ... after tryptophan l... children with and ... out fits and not recei... phenytoin and in corr sponding groups recei ing phenytoin The upp regression line refer to the children who ... receiving phenytoin and the lower one to the children who ... not. The regression coeffi cients are significant ($p < 0.01$).

leptic children) were small and insignificant (Fig. 1 Table 1). Nor did the epileptic children on phenytoin differ in this respect from the apparently healthy children receiving the same dose of phenytoin.

The results of the tryptophan load tests performed in the same children before and during the administration of phenytoin are summarized and analyzed in Table 2. In 7 of 8 epileptic children and in all 5 apparently healthy children the excretion of xanthurenic acid was higher before than during the administration of phenytoin. The mean difference for the entire group (including both apparently healthy controls and 8 epileptic children) was significant ($p < 0.0025$). Addition of phenytoin to urine samples did not alter

Table 1 *Xanthurenic acid excretion during 7 hours after the administration of 0 1 g L-tryptophan/kg in healthy children, in children with epilepsy and other neurological disorders and not receiving hydantoin derivatives and in children receiving phenytoin (10 mg/kg/day)*

	N	Mean age	Excretion of xanthurenic acid (µmoles/kg body wt/7 hours) Range	Mean	S.D.
I. *Children aged 0–2 years*		Months			
(*a*) Apparently healthy	5	11	0 30–1 18	0 55	0 36
(*b*) With neurol. disorders, no epilepsy	2	11	0.39–0.57	0.48	—
(*c*) With untreated epilepsy	8	7	0.11–0 81	0 43	0.24
(*d*) With epilepsy treated without hydantoin	4	12	0 19–0 80	0.51	0.25
(*e*) Apparently healthy receiving phenytoin	1	18	3.11	3 11	—
(*f*) With epilepsy treated with phenytoin	5	11	0.28–2 35	1.23	0 85
II. *Children aged 2 15 years*		Years			
(*a*) Apparently healthy	9	7½	0 49–1.86	1 16	0 45
(*b*) With neurol disorders, no epilepsy	8	6½	0.47–3.07	1.20	0.81
(*c*) With untreated epilepsy	6	8½	0 66–2 43	1.25	0.65
(*d*) With epilepsy treated without hydantoin	3	7	0.83–1 98	1.27	—
(*e*) Apparently healthy receiving phenytoin	4	7	1 48–2.97	2.26	0 61
(*f*) With epilepsy treated with phenytoin	10	6½	0 35–3 36	1.91	[illegible]

Table 2. *Increase of xanthurenic acid excretion in patients with epilepsy and control subjects before and during phenytoin administration*

Subjects	Age	Xanthurenic acid excretion (µmole/kg/7 hours) Before phenytoin	During phenytoin	Difference	p
Patients with ep					
1	6 m.	0.63	0.87	0.24	
	11 m.	0.62	0.78	0.16	
3	14 m.	0.19	2.35	2.16	
4	17 m.	0.36	1.85	1.49	
5	5½ y	1.16	0.78	−0.38	
6	8 y	0.68	3.36	2.68	
7	11½ y	2.43	2.90	0.47	
8	12 y	0.66	1.50	0.84	
Mean of 8 pat.	5 y	0.84	1.80	0.96	<0.02
Controls					
1	18 m.	1.18	3.11	1.93	
2	4½ y	0.68	1.48	0.80	
3	6½ y	0.81	2.97	2.16	
4	8 y	1.47	2.36	0.89	
5	8½ y	1.54	2.22	0.68	
Mean of 5 controls	6 y	1.14	2.43	1.29	<0.02
Overall mean	5½ y	0.95	2.04	1.09	<0.0025

the xanthurenic acid content, as measured with the method used.

DISCUSSION

In a preliminary report (5) a correlation was found between increased excretion of xanthurenic acid after tryptophan loading and the administration of hydantoin derivatives. That report was based on a material of 69 individuals, 46 of whom had fits, studied by a somewhat different method of xanthurenic acid determination. The xanthurenic acid excretion found in the controls of the present investigation is in agreement with that reported by other investigators (4 8 10) Increasing xanthurenic acid excretion (related to body weight) with age has also been reported by others (3 4 11)

Owing to the heterogeneity of the material the differences between the results of the tryptophan load tests in the children with various disorders receiving phenytoin and those not receiving phenytoin cannot be accepted as a definite proof of an augmenting effect of phenytoin on the excretion of xanthurenic acid. The study was therefore extended with tryptophan load tests on healthy children. Judging from the results in Table 2, the drug is responsible for the elevation of the xanthurenic aciduria. Methyl-phenylaethyl hydantoin (Mesantoin®) had the same effect in our previous material phenytoin was the only hydantoin derivate used in the present study

Other investigators have also discussed the possible effect of antiepileptic treatment, particularly phenytoin, on the tryptophan load test. In a study on infantile spasms, French *et al* (4) found the tryptophan metabolism to be disturbed in a case of phenytoin intoxication and they tested 6 normal children after seven days' administration of phenytoin (5 mg/kg/day) with negative results. Nevertheless, of 6 of their patients with infantile spasms and abnormal tryptophan metabolism 5 received phenytoin, whereas of 9 patients with normal tryptophan metabolism only 3 were treated with phenytoin. They received about 7 mg/kg/day Hagberg *et al.* (9) found abnormal tryptophan load tests somewhat more often among the patients on phenytoin than among the rest of the patients, but the difference was not significant. Hughes *et al* (11) also failed to demonstrate any influence of anticonvulsants on tryptophan metabolism. Several possibilities may be offered to explain the conflicting results.

1 *Difference in method* Hagberg *et al* (9) and

Hughes *et al* (11) used a colorimetric method with ferric ammonium sulphate for estimating xanthurenic acid. This method is less specific than the chromatographic separation used in our study Hughes *et al* (11), however who used both a ferric ammonium sulphate method and a chromatographic technique in many of their patients, found good agreement between the results obtained with the two methods. Hence the use of different chemical methods cannot be the only explanation for the discrepancies between our results and those obtained by others. Moreover French *et al* (4), who used a method which was essentially the same as ours, were also among the investigators who failed to demonstrate any effect of phenytoin on the tryptophan load test.

_. *No allowance for age differences* of the children in the determination of the excretion of tryptophan metabolites. This source of error is difficult to evaluate because some of the investigators have not given the individual ages or xanthurenic acid excretion values for patients receiving and those not receiving phenytoin. The results of Hagberg *et al* (9) do not seem to be biased in this respect. Their patients receiving hydantoin derivatives were actually a little older than those not receiving such derivatives.

3 *The dose of phenytoin* An optimal anticonvulsive effect and normalization of the EEG requires a serum phenytoin level of 10–20 μg/ml. In children weighing less than _5 kg achievement of this level requires administration of 10–15 mg phenytoin/kg/day In children above this weight the dose can gradually be lowered to 6 mg/kg/day which is adequate for adults (15). This policy is followed in our treatment of epileptic children. French *et al* (4) gave 7 mg/kg/day of phenytoin to their patients and 5 mg/kg/day to their control children, aged 6– 4 months. Particularly the latter dose is probably too small to affect the tryptophan metabolism. Other uthors have not stated the amount given and Hughes *et al.* (11) reported neither the dosage nor the type of anticonvulsive medication. The main discrepancy between our results and those obtained by other investigators can perhaps be explained mainly by differences in the dose of phenytoin used, and to some extent by the lack of due consideration to the influence of age on the tryptophan load test.

The finding that phenytoin added to the urine samples did not cause an increase in the estimated xanthurenic acid content excludes th possibility of the presence of the drug in the urin giving a false high xanthurenic acid level. It cannot be excluded that some metabolites of phenytoin may cause such a disturbance. In view of good specificity of the method, however it is more likely that phenytoin causes a true elevation of xanthurenic acid excretion.

During the past 3 years large doses of pyridoxine were tried on many of our patients with frequent fits (including several children with infantile spasms). Only in occasional patients was a definite response seen. In these patients the tryptophan load test was normal before treatment with pyridoxine. One of them might possibly be classified under the heading of pyridoxine dependency (*symptoms starting before 1 month of age*) In contrast to some other investigators (9 11) we have so far encountered no instance of marked xanthurenic aciduria.

Som investigators have reported that the tryptophan metabolism is disturbed more often in patients with *cryptogenous* epilepsy than in patients with *symptomatic* epilepsy (1 9). This seemed to fit with the assumption that in the cryptogenous group hidden metabolic cause (with in some cases also involvement of the tryptophan metabolism) ought to be more frequent than in the symptomatic group, despite the obvious difficulties in dividing a clinical material in these two etiological groups. On analyzing the present material in this respect, no difference was seen in the excretion of xanthurenic acid. Bower (1), who first reported the higher incidence of increased excretion of xanthurenic acid in cryptogenous epilepsy recently concluded that his observation could not be confirmed in a larger series (11) We agree with these authors that the tryptophan load test is rarely of any practical value. It is nevertheless wortwhile to examine the tryptophan metabolism (during and without pyridoxine medication) in patients with frequent fits and/or mental stagnation, who improve on pyridoxine administration Further metabolic in estigations in such cases (6, 7) may contribute to a better understandi g of these still obscure pyridoxine-responsive abnormalities. I evaluation of the results of the tryptophan load test the age of the patient and the effect of various drugs must, however be taken into account.

SUMMARY

Tryptophan load tests were performed on 51 children, including 27 with epilepsy In controls and in patients not receiving phenytoin the xanthurenic acid excretion in urine increased with age. No difference was found between the mean values for controls, non-epileptic patients with neurological diseases and epileptic children not receiving hydantoin derivatives. In epileptic children treated with phenytoin the excretion of xanthurenic acid was higher than in the other 3 groups. The xanthurenic acid excretion increased in healthy children given phenytoin 10 mg/kg/day for at least 5 days. The mean difference between the results of the tryptophan load test before and during phenytoin administration in 8 epileptic children and 5 healthy controls was significant. In all but one of these children xanthurenic aciduria increased during administration of phenytoin. In none of the few patients who showed a clinical response on pyridoxine (100–150 mg/day) was the tryptophan load test abnormal before treatment with pyridoxine.

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DEVELOPMENT OF UREA SYNTHESIZING ENZYMES IN HUMAN LIVER

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Although a large number of studies on enzyme development in various tissues of experimental animals has accumulated during recent years, very few investigations have been concerned with human embryos. The data obtained from laboratory animals cannot be applied direct to human subjects, since marked differences can occur between species. If a better understanding of both normal and pathological development of the human foetus and neonate is to be gained, such studies should also relate to human material.

Studies by Kennan & Cohen (4), and by Räihä & Kretchmer (9), have shown that the urea cycle in the rat is not active before birth, but that soon afterwards the capacity for urea biosynthesis increases to adult levels. Only two reports on direct measurements of developing human liver tissue have been found, those made by Manderscheid (5) and by Kennan & Cohen (4): they observed urea production in liver slices from human foetal liver of 3 to 4 months gestation. The present study provides data on the activities of the enzymes involved in urea synthesis in liver homogenates, and on the urea-synthesizing capacity of liver slices during human development.

MATERIAL AND METHODS

Human Liver Tissue. Human foetal liver was obtained from legal abortions. The gestational age of the foetuses ranged between 3 and 25 weeks, and was estimated from the weight and crown rump length. The liver from the postnatal and adult subjects was obtained by open biopsy during abdominal surgery. None of the patients from whom liver tissue was obtained suffered from disease involving the liver except for the two newborn infants subjected to surgery following rupture of the liver.

The liver tissue was frozen immediately in CO_2-ice, and 10 % homogenate was prepared in cold 0.1 % cetyltrimethylammonium bromide (CTB) for the enzyme determinations.

Urea synthesis by liver slices. The actual overall rate of urea synthesis was studied in slices from foetal human liver immediately after excision of the liver tissue.

Slices with uniform thickness of 0.3 mm were cut in the cold room by means of a transparent pre-chilled plastic block (8 cm × 5 cm × 1 cm), with a section (8 cm × 1 cm × 0.03 cm deep) cut from the surface. The cold block, moistened with chilled buffer, was placed on the sample of liver and slices were cut by sliding a cold razor blade against the raised edges of the block. Slices approximately 100 mg in weight were blotted dry and immediately transferred to weighed 25 ml glass stoppered Erlenmeyer flasks containing the incubation medium. The incubation medium consisted of 3.0 ml Krebs-Ringer-bicarbonate buffer gassed with 95 % O_2 + 5 % CO_2, containing 200 µmoles of NH_4Cl, 1 µmoles of L-ornithine, 60 µmoles of DL-lactate, 28 µmoles of glucose.

The flasks were reweighed to arrive at the weight of the slices added, and gassed 95 % O_2 + 5 % CO_2. 5–10 10^6 counts/min of ^{14}C-bicarbonate was added; the flasks were stoppered, and incubated for 60 minutes at 37°C in a Dubnoff incubator. The reaction was stopped with 100 µl of 2 N HCl, and the contents of the flasks were homogenized. To eliminate any dissolved radioactive CO_2 the homogenate was flushed with CO_2. Experiments with boiled liver slices showed that all the dissolved radioactive CO_2 could be removed in this way. The homogenate was neutralized with 2 N NaOH, and 1 ml aliquots were placed in Warburg flasks containing urease in the side arm, and then sealed with serum bottle stoppers. The urease was tipped in, and the CO_2 liberated during 60 minutes of incubation, with shaking, was trapped in 0.5 ml of Hyamine Hydroxide (10-X) kept in a small glass cup in the closed flask. Dissolved CO_2 was liberated by the injection of 2 N HCl into the medium at the end of the incubation. The radioactive CO_2 trapped in the Hyamine was counted in a Packard liquid scintillation counter. Control experiments made with ^{14}C

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that 100% of the radioactive carbon was recovered by this technique The results have been expressed as the percentage of ^{14}C-bicarbonate incorporated into urea per 100 mg liver wet weight per hour

Estimation of enzyme activities The enzymes of urea synthesis, carbamyl phosphate synthetase (EC 2.7.2.2.), ornithine transcarbamylase (EC 2.1.3.3.) argininosuccinase (EC 4.3.2.1.) and arginase (EC 3.5.3.1.) were determined by application of the techniques described by Brown & Cohen (1). The activity of the arginine synthetase system (overall reaction of Ratner (7)) was also determined by a modified form of technique described by Brown & Cohen (1). The assay system comprised 1.25 μmoles of L-ureido-^{14}C-citrulline (New England Nuclear Corporation, Boston Mass., specific activity 1.8 10^6 cpm per μmole), 1.25 μmoles of -aspartate, 1.25 μmoles of ATP 1.25 μmoles of $MgSO_4$, 12.5 μmoles f potassium phosphate buffer (pH 7.8) and an excess of arginase (Worthington Biochemicals Co.) The total volume was 300 μl, and 50 μl of the tissue extract was used. The incubation was continued for 20 minutes at 37 C, and stopped by boiling the tubes for 3 minutes. The reaction mixture was transferred into sealed flasks, and urease added to liberate the carbon dioxide from the urea formed. The liberated carbon dioxide was trapped in Hyamine hydroxide and the radioactivity counted in a Packard Tri-Carb liquid scintillation counter

Experiments with different concentrations of citrulline, ATP and 1-aspartate, with both foetal and adult liver preparations, demonstrated that the concentrations used in the assay gave maximum arginine synthetase activity

As the level of argininosuccinase (EC 4.3.2.1.) is higher than that of argininosuccinate synthetase (EC 6.3.4.5.) in human liver (present study) and in rat liver (6, 10), the system applied measures the activity of the latter

Enzyme Units. Enzyme activities were calculated as the amount of enzyme which catalyses the production of one μmole f product per hour under assay conditions. Enzyme activities are expressed as units per g liver wet weight. The protein content of the liver CTB homogenate was estimated by the method of Lowry *et al.*, it is observable from F g. 1 that very little variation in protein content was found during development.

RESULTS

The overall urea production was studied in liver slices from 5 human foetuses with a gestational age varying from 16 to 20 weeks. All the livers studied produced urea, and the mean urea-producing capacity was 0.34% (range = 0.26% – to 0.48%), expressed as a percentage of the ^{14}C bicarbonate incorporated into urea per 100 mg liver per hour This corresponds to about 4 μmoles/g/hour and is the same as that found in a rat at birth (9). In the rat and guinea pig mid way through gestation, no urea producing capacity is detectable (9).

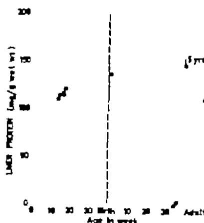


Fig 1 Protein content of the human liver during development.

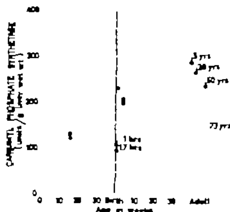


Fig 2. Carbamyl phosphate synthetase activity of the liver during human development.

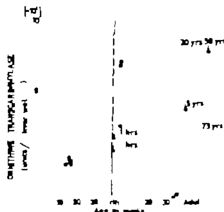


Fig 3 Ornithine transcarbamylase activity of the liver during human development.

The developmental pattern of the urea-synthetizing enzymes in human liver is indicated in Figs. 2 to 5 No postparturition increase in activity is apparent in any one of the five enzymes studied. In general, the enzyme activities in the foetal and

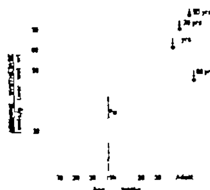


Fig. 4 Arginine synthetase activity of the liver during human development.

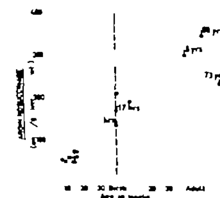


Fig. 5 Argininosuccinase activity of the liver during human development.

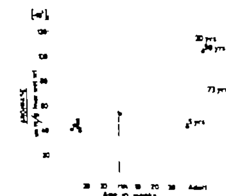


Fig. 6 Arginase activity of the liver during human development.

newborn livers are slightly lower than those found in the adult livers, although considerable activity is present even at a ery early foetal stage. The arginine sy thetase system has the lowest activity: the mean alue is 64 units per g liver in adult human liver. The apparent Km values or citrulline, as estimated from the Lineweaver–Burk plot for the arginine synthetase system, gave a value of $2.8 \times 10^{-4} M$ for adult enzyme preparations, and $2.0 \times 10^{-4} M$ for foetal.

DISCUSSION

The present study makes it apparent that the developmental pattern of the urea synthesizing enzymes in the human is different from that found in the rat (4 9). In the rat, urea is not synthesized until around birth. In the pig, how ever Kennan & Cohen (4) found a pattern similar to that found in the human in the present study These authors found that the enzymes were present at significant levels in the liver of a pig embryo studied at 28 days, and discussed the species difference between the rat and the pig in relation to the foetal membranes of the two animals, and the maturation of the foetus and the newborn.

Edelman *et al.* (2) and Sunshine & Kretchmer (11) have demonstrated that premature infants are able to increase their blood urea-N and urea excretion when a high protein diet is administered. Further Friedberg (3) has found a passage of urea via the urethra, with some accumulation in the amniotic fluid during the last four months of pregnancy These findings are in agreement with the present results, which indicate that the human foetus is capable of synthesizi g urea at an early stage of gestation. The arginine synthetase system has the lowest relative activity and is thus also in human liver the rate-limiting enzyme of the urea cycle.

The Km values for citrulline in the arginine synthetase reaction are similar in magnitude in both foetal and adult human liver enzyme pre parations, and compare well with the values reported by Ratner (8) for rat liver enzyme, and by Tedesco & Mellman (12) for the enzyme in cul tured human fibroblasts.

This study supports the view that data obtained from experimental animals should not be applied direct to human development.

SUMMARY

Li er slices from human foetuses of a gestational ge between 16 and 20 weeks produce urea when incubated w th optimal amounts of substrate.

The activities of the enzymes which synthesize urea (carbamyl phosphate synthetase, ornithine transcarbamylase arginine synthetase system, argininosuccinase and arginase) have been measured in human liver during development. No rapid postparturition increase in activity was discernible in any of the enzymes studied, although in general the adult levels were somewhat higher than those found at birth. Arginine synthetase has the lowest relative activity of all the five enzymes involved in urea synthesis. The apparent Km for citrulline is similar in magnitude for both foetal and adult liver arginine synthetase.

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STUDIES ON ERYTHRO-KINETICS IN INFANCY

IX. *Prediction of Red Cell Volume from Venous Haematocrit in Early Infancy*

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The circulating red cell volume (RCV) constitutes an important parameter in clinical haematology. The use of this parameter is largely restricted by the fact that its determination is rather complicated. This is particularly true for determinations in infants, and there is thus a need for a prediction formula valid for infants. The RCV prediction should be based upon parameters that are easily observed, and reasonable precision in this prediction is desirable.

Several formulas have been worked out for prediction of the blood volume in adults (1 5 7 8, 11 19 27), children and infants (4 6, 7 14 18, 21 22), based on body weight, height, or combinations of these two and sometimes also together with other anthropometric parameters such as skin fold thickness and skeletal size. However formulas of the types mentioned are not suitable for prediction of RCV in early infancy. During the first months of life the RCV decreases at the same time as other body parameters (e.g. weight, height) are increasing. In fact, no prediction formula has been proposed expressively for the first 4 5 months of age.

A basis for prediction of RCV in this age group is given by the correlation between RCV/kg body weight and venous haematocrit (vHct). In adults, this relationship has been described by many authors (2, 12, 15 20). In newborn infants the correlation has been studied by Mollison *et al.* (16), in three-day old infants by Usher *et al* (25), in 1-94-day old premature infants by Schulman & Smith (22) and in 1 5-day old premature infants by Usher & Lind (26). In these studies a fairly close relationship between RCV/kg and vHct was found. On the other hand, Sisson *et al* (23) found no statistically significant correlation between RCV/kg and vHct in full-term infants between birth and one year of age. Furthermore, Sisson *et al* (24) found only a poor relationship in a study on premature infants of the same age. Only Mollison *et al* (16) measured the RCV while the other authors mentioned calculated this volume from data of plasma volume (PV) determinations.

In view of these contradictory findings and, especially the reported lack of relationship in full-term infants over a long age interval, the problem was reinvestigated in infants between birth and 5 months of age.

MATERIAL AND METHODS

Sixty-six determinations of RCV were performed, using ^{51}Cr labelled autologous red blood cells according to method described previously (3). With this technique, the error of a single measurement is $\pm 3\%$. 40 of the measurements were performed on 24 full-term normal infants aged 9 hours to 138 days, and 26 on 16 normal premature infants aged 4 hours to 98 days. The venous haematocrit was determined, using an International Micro-capillary Centrifuge, Model MB. With this centrifuge the amount of trapped plasma is 1.7% after 3 minutes centrifugation and 1.3% after 10 minutes' centrifugation (10). The centrifugation time in this study was 5 minutes. Correction was made for an assumed amount of 1.5% of trapped plasma.

The regression of RCV on vHct was calculated by standard methods of least squares. It appeared that the variance of RCV increased with increasing vHct values. Since prediction limits of RCV at various vHct values were aimed at, the variation around the regression line was estimated. This was done by studying the relationship between the squared RCV-residuals around the regression line and the corresponding vHct values. This relationship was assumed to be linear and was estimated by means of ordinary regression analysis.

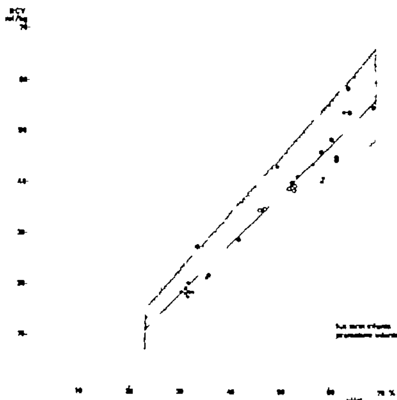


Fig. 1. Correlation between red cell volume/kg body weight and venous haematocrit in 40 infants aged 4 hours to 138 days. The slope of the regression line, $y = -12.3 + 1.02x$, is statistically highly significant. The shaded area represents the confidence limits of ± 2 s.D.

RESULTS

The relationship between the individual values of RCV/kg and vHct is shown in Fig. 1. The results of simple linear and second degree polynomial regression analyses are shown in Table 1. The equations of simple linear regression are shown in Table 2. Assuming homoscedasticity the standard deviation about the linear regression line referring to full-term + premature infants is 3.6 ml/kg. As seen from the figure and as mentioned before, the variance, however, is not homogeneous, but increases with larger values of vHct (i.e. x). The residual variance (σ^2_{RCV}) in RCV can be expressed approximately as a function of vHct as follows: $\sigma^2_{RCV} = -7.23 + 0.43$ vHct. This equation was used when deriving the prediction limits in Fig. 1. The above relationship could be used in a weighted regression analysis of RCV on vHct. This was not considered worth while, however, on these data. The calculated standard deviation, which is thus a function of x, varies between 1.1 and 4.7 ml/kg, i.e. 8-14% of the predicted RCV/kg (i.e. y). The fact that results of repeated measurements on the same infants were included in this study may have had some effect on the estimation of the standard deviation. The straight line character of the relationship between RCV/kg and vHct in this material is shown by the fact that the variance around the second order polynomial regression line is almost as large as the variance around the linear regression line, and that the second degree polynomial line (RCV/kg $= -3.3 + 0.59$ vHct $+ 0.0047$ vHct^2) is almost straight (Table 1, cf. also Fig. 3).

DISCUSSION

The equations of the linear regression between RCV and vHct from the studies of Mollison *et al.* (16), Schulman & Smith (22), Usher *et al.* (25), Usher & Lind (26) and the present author are shown in Table 2. The regression lines of these equations are shown in Fig. 2. The correlations between RCV/kg and vHct in infants given by the different authors are fairly similar. Some dissimilarities might be expected as a result of different techniques used by the authors and by the fact that the range and distribution within the range of the vHct used in these calculations differ.

Mollison *et al.* (16) and the present author

Table 1 *Regression analysis of relation between venous haematocrit (x) and red cell volume/kg (y) in early infancy*

	Full-term infants			*Premature infants*			*Full-term + premature infants*		
Sample size	40			26			66		
Range of *x*	27.58–67.47 (mean 44.01)			23.64–64.02 (mean 50.39)			23.64–67.47 (mean 46.52)		
Range of *y*	17.34–55.16 (mean 32.00)			14.97–58.90 (mean 39.62)			14.97–58.90 (mean 35.00)		
REGRESSION OF ONE INDEPENDENT VARIABLE									
Intercept	−11.135			−13.365			−12.275		
Regression coefficient	0.9800			1.0514			1.016		
Standard error of regr. coeff.	0.0451			0.0711			0.037		
Correlation coefficient	0.962			0.949			0.960		
Analysis of variance for simple linear regression									
Source of variance	Degree of freedom	Sum of squares	Mean square	Degree of freedom	Sum of squares	Mean square	Degree of freedom	Sum of squares	Mean square
due to regression	1	5691.36	5691.36	1	3116.67	3116.67	1	9691.82	9691.82
deviation about regression	38	457.28	12.03	24	341.72	14.24	64	830.57	12.98
total	39	6148.64		25	3458.39		65	10,522.39	
POLYNOMIAL REGRESSION OF SECOND DEGREE									
Intercept	−7.269			3.321			−3.900		
Regression coefficients	0.7942	0.0021		0.2924	0.0081		0.5909	0.0047	
Standard error of Regression coefficients	0.4352	0.0048		0.5530	0.0059		0.3355	0.0037	
Analysis of variance for second degree polynomial									
Source of variation	Degree of freedom	Sum of squares	Mean square	Degree of freedom	Sum of squares	Mean square	Degree of freedom	Sum of squares	Mean square
due to regression	2	5693.62	2846.81	2	3142.92	1571.46	2	9712.72	4856.36
deviation about regression	37	455.02	12.30	23	315.46	13.72	63	809.67	12.85
total	39	6148.64		25	3458.39		65	10,522.39	

made measurements of RCV Mollison *et al.* with a ^{32}P and a differential agglutination method and the present author with a ^{51}Cr technique. These three methods may be regarded as equivalent (16, 17). In both of these two studies correction was made for trapped plasma. The linear relationship between RCV/kg and vHct (in the range 39.9–66.2%) in normal full-term infants calculated from the work of Mollison *et al.* (16) is indicated by the straightness of the second degree polynomial line, $RCV/kg = -17.6 + 1.22\ vHct - 0.003\ Hct^2$ (Fig 3), and by the almost identical residual variations of the linear and second degree polynomial regressions. The slope and the position of the linear regression line of Mollison *et al* (16) are not statistically significantly different from those referring to full term infants of the present study. The regression equation of Mollison *et al* is based on measurements in younger infants (approximately 70% of them younger than 6 hours) than any other equation discussed in this paper. During the first hours of life there is an increase in vHct due to fluid shift (9, 25). This fact makes prediction in this period less precise and may explain the relatively low correlation coefficient of Mollison *et al* (cf Table 2).

Schulman & Smith (22), Usher *et al* (22, 26) and Sisson *et al.* (23, 24) made no RCV measurements but calculated RCV from data of PV determinations by use of the body/venous haematocrit ratio 0.87 proposed by Mollison *et al* (16). This ratio is valid as a correction factor if it is used in the same way as it was obtained by Mollison *et al* who performed their PV measurements on newborn infants with a T 1824 technique and based their calculations on a blood sample withdrawn 10 minutes after injection without correction for loss of the tracer before sampling. If PV measurements are performed in a different way or on infants differing (e.g. with re-

Table 2. *Correlation between venous haematocrit (x) and red cell volume/kg body weight (y)*

Authors	Full term or prematures	Age (days)	Number of deter minations	Range of venous haematocrit	Equation	Correlation coeffic.
Mollison, Veall & Cutbush (1950)	Full term	0	32[a]	39.9–66.2	(I) $y = -9.54 + 0.909x$[b]	0.908
Schulman & Smith (1954)	Prematures	1–94	38	22.2–66.7	(II) $y = -11.2 + 1.04x$	0.91
Usher Shephard & Lind (1963)	Full term	3	26	38.5–67.0	(III) $y = -10.08 + 0.9603x$	
Usher & Lind (1965)	Prematures	1–5	25	About 40–76	(IV) $y = -22.35 + 1.232x$	
Present study	Full term	0–138	40	27.6–67.2	(V) $y = -11.13 + 0.980x$[b]	0.962
Present study	Prematures	0–98	26	23.6–64.0	(VI) $y = -13.36 + 1.051x$[b]	0.949
Present study	Full term + prematures	0–138	66	23.6–67.2	(VII) $y = -12.28 + 1.016x$[b]	0.960
Huber, Lewis & Szur (1964)		Adults	159[c]	23.4–65.3	(VIII) $y = -15.39 + 1.064x$[b]	0.924

[a] Only including the normal, full-term infants of their study (31 cases estimated by the ³²P-technique and one by the Ashby method).
[b] Calculated by the present author.
[c] Correction for 1.5 % of trapped plasma made by the present author. 159 cases selected with the same range of corrected Hct as the infants studied by the present author.

spect to age) from those studied by Mollison *et al.* (16), strictly correct estimations should be based on PV measurements where correction is made for the disappearance of tracer from the circulation. In newborn infants the disappearance of I labelled human serum albumin is approximately 6 % during the first 10 minutes following injection (3). From such a PV estimation the RCV should be calculated by use of a body/venous haematocrit ratio valid for PV estimations corrected for loss of tracer before sampling. This body/venous haematocrit ratio is 0.91 not only in the adult, but also in the newborn infant (3).

Schulman & Smith (22) made PV measurements on premature infants aged 1–94 days essentially according to the principles used by Mollison *et al.*

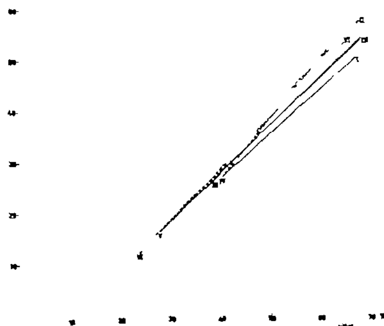


Fig. 2. Correlation between venous haematocrit (x) and red cell volume/kg body weight (y) in infants. The regression lines refer to equations shown in Table 2.

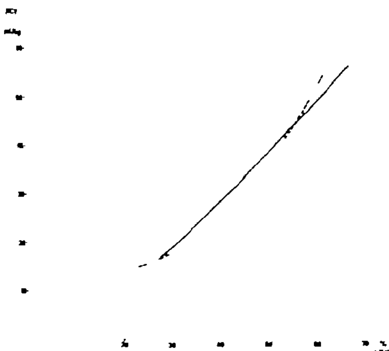


Fig. 3. The second degree polynomial regression lines of the relationship between red cell volume/kg body weight and venous haematocrit based on the work of Mollison *et al.* (16) on full-term newborn infants (——), the present study on full-term infants less than 5 months of age (———) and the work of Huber *et al.* (12) on adults (——). The equations of these lines are given on pages 127 and 130 and in Table I.

(16). As Mollison *et al.* studied mainly full-term infants during the first day of life it is not quite certain that the correction factor 0.87 used by Schulman & Smith (22), is valid in premature infants, and after the first day of life.

Usher *et al.* (25) performed PV measurements on full-term newborn infants with [131]I-labelled albumin using a Volemetron method. One 5 minute sample was used in each infant. No correction was made for disappearance of the label before sampling. These authors calculated RCV using the correction factor 0.87 which ought to result in a slight overcorrection for label loss, since Mollison *et al.* (16) based their calculations on a 10 minute sample. In newborn premature infants Usher & Lind (26) used the same technique as Usher *et al.* (25) and here, as for Schulman & Smith (22), the reservation should be made that the correction factor 0.87 was not obtained in a study on prematures.

In the studies of Usher *et al.* (25) and Usher & Lind (26) no correction of the haematocrit readings was made for trapped plasma (about 2 % (25)). A total correction of the data of Usher *et al.* (25), including correction for sampling at 5 instead of at 10 minutes and correction for trapped plasma means that their RCV values should be multiplied by a factor with a range from 1.02 at a vHct of 40 to 1.08 at a vHct of 70 and that their vHct values should be reduced by 2 %. The equation of the relationship between RCV/kg and vHct after this correction will be: $y = -14 + 1.10\ x$. If the corresponding corrections are made on the data of Usher & Lind (26) the new equation will be $y = -37 + 1.6\ x$. The fact that the original regression lines of Usher *et al.* (25) and Usher & Lind (26) are almost identical with that of Mollison *et al.* and the regression lines of the present author seems to imply that the disappearance of the tracer of Usher *et al.* (25, 26), i.e. [131]I-labelled albumin, in 5 minutes, was approximately as large as the disappearance of the tracer of Mollison *et al.* (16), i.e. T 1824, in 10 minutes. The explanation for this could be that some of the [131]I-labelled albumin used by Usher *et al.* (25, 26) was denatured (cf. also discussion of the work of Jegier *et al.* by Bratteby (3)). If this interpretation is correct, the values of RCV calculated by Usher *et al.* (25, 26) are accurate.

Usher & Lind (26) found that the slope of the regression line for the relation between RCV and vHct in prematures was not significantly different from that obtained for full-term infants by Usher *et al.* (25). However, the positions of these lines

were different. It is possible that part of this difference is due to the different ranges of vHct in these two studies. In the work of the present author neither the slopes nor the positions of the regression lines are statistically significantly different when prematures and full-term infants are compared.

Simon *et al* (23) studied the blood volume in 126 full-term infants, aged 0 days to 52 weeks, using a T 1824 technique. In this work no relationship was found between RCV and vHct. Sisson *et al* (24) studying 51 premature infants aged 2 days to 51 weeks, with the same technique, found only a weak correlation ($r = 0.54$) between RCV/kg and vHct. The poor correlations in these two studies are possibly due to methodological errors in the RCV estimations. From the studies referred to earlier it seems evident that a close relationship does exist between RCV/kg and vHct in the newborn infant. The investigations of Schulman & Smith (22) and of the present author show that this correlation is present in premature and full-term infants during the first four to five months of life. It is most probable that the relationship exists even beyond these first five months.

Huber *et al* (12) studied the relationship between RCV and vHct in adults. RCV was measured with a ^{51}Cr technique, and a microhaematocrit method with 5 minutes centrifugation at approximately 12,000 × *g* was used without correction for trapped plasma. The individual values of vHct and RCV/kg from 200 patients without a major degree of splenomegaly and with a vHct range of 13.8 73 8% were kindly offered by these authors for comparison with the present data from infants. The values of RCV and vHct were corrected for an assumed amount of 1.5% of trapped plasma. Regression analysis was then performed on the values of 159 patients with a range of corrected vHct from 23.39 to 65.25%. The equation of the correlation between RCV/kg and vHct is shown in Table 2. The simple linear relation between RCV/kg and vHct in adults is fairly similar to that found in infants. However in these adults, a slightly better fit was given by a second degree polynomial, $RCV = 19.6 + 0.65\ vHct + 0.019\ vHct^2$ (Fig. 3) In this connection it must be mentioned that there is a restriction in the comparison between infants and adults. In infants the vHct shows a rather wide physiological range during the first months of life whereas in the adult this range is rather narrow. Wider boundaries can be obtained in the vHct of the adult only by including pathological cases. In spite of this it would be of great interest to find out whether the same relationship between RCV/kg and vHct exists in all age groups.

For practical purposes, RCV in full-term and premature infants from the fourth hour to the fifth month of life may be predicted from vHct in the range 23–67%, using the following equation. RCV/kg = vHct 12. Assuming no error in the determination of vHct the standard error of estimate will be approximately 10% of the predicted RCV. This error is in the same range as those obtained for most formulas suggested for prediction of blood volume in adults (1 5 7 8, 11 27).

SUMMARY

Sixty-six determinations of circulating red cell volume (RCV) were performed in 24 normal full-term and 16 normal premature infants aged 4 hours to 138 days. The correlation between venous haematocrit (vHct) and RCV/kg was analysed. Within the range of vHct studied, 23–67%, the relationship was linear according to the equation. $RCV/kg = -12.3 + 1.02\ vHct$. No significant difference in slope or position of the regression lines was found between the full-term and premature infants. A comparison with adult individuals, taken from the literature showed an almost identical relationship. The equation is proposed as a formula for prediction of RCV in full-term and premature infants during the first five months of life. The standard error of estimate for prediction from this formula is approximately 10%.

ACKNOWLEDGEMENTS

My thanks are due to Dr. Lars Garby and Mr Adam Taube, lecturer in statistics, for advice and criticism. I am indebted to Drs. Huber Lewis and Szur The Post-graduate Medical School, London, who placed their results of RCV and vHct estimations in adults at my disposal.

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Table 1 *Gestational age birth weight age at time of investigation and haematocrit and red cell volume*

Case	Gestational age (days)	Birth weight (g)	Body length (cm)	Age[a] (days)	Venous haematocrit ( )	Red cell volume (ml)
1	222	1660	42	3	46.3	54 1
2	230	1960	43	1	50.7	86 4
3	235	1920	44	2	62 0	10?
4	235	1690	43	0	44 1	44.7
5	238	2020	44	1	5_0	75 5
6	242	2370	46	1	51.0	90 8
7	243	1970	42	2	60.1	82.5
8	45	1950	45	2	49.2	76 6
9	45	100	46	1	51 7	94 1
10	247	2400	45	0	64 0	130
11	249	2300	46	0	61.8	108
12	252	2190	46	2	61.3	120
13	260	2620	48	1	48.5	106
14	261	2680	47	2	67.5	138
15	261	2370	48	1	62.5	120
16	263	3410	50	0	55 6	152
17	264	2230	47	3	60 1	93 9
18	270	3720	51	2	67.2	191
19	273	4500	53	0	54.2	170
20	279	4070	53		46.8	155
21	282	3130	48	3	57.9	121
22	285	3710	50	2	52 2	150
23	288	4290	54	0	57 6	169
24	291	3670	50	1	53 2	155
25	292	3410	50	3	52 2	120
26	292	4510	51	1	57.6	178
27	295	3810	51	2	52.7	150

[a] Referring to date of birth.

is shown in Fig 4 At a gestational age of 240 days (±14 days) a value of 43.8 ml kg birth weight was obtained, and at 280 days (±14 days) a value of 40.3 ml/kg.

DISCUSSION

A similar relationship between birth weight and gestational age (Fig. 2) was found in the studies of Brody & Nilsson (4), Usher *et al* (11), Usher & Lind (12) and the present author 83 of the infants of different gestational ages from these studies had birth weights within the confidence limits of 10 and 90 percentiles, suggested by Engström & Sterky (5) and 94% of them were within the 5–95 percentile range of Hosemann (8). A mean feature, however is that infants of a gestational age of less than 60 days had birth weights which were less than the mean reported by Engström & Sterky (5). On the other hand the birth weights of these infants of different gestational ge showed better fit with the mean expected birth weights after different lengths of intrauterine life published by Hoseman (8) Gruenwald & Minh (7) and Lubchencho *et al.* (9) It is possible that the infants from the studies of Brody & Nilsson (4), Usher *et al* (11 and 1) and the present author are not quite representative of all infants born after different gestational pe-

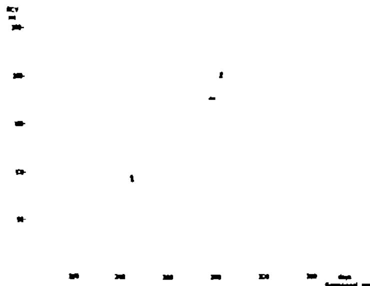


Fig. 1 The relationship between red cell volume and gestational age in 27 normal infants studied by the present author (●) by [51]Cr-dilution method compared with data from 43 plasma volume measurements by Brody & Nilsson (4) (□), and Usher *et al.* (11) and Usher & Lind (12) (△). Values from the two latter studies represent the mean of measurements performed within 72 hours after birth.

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STUDIES ON ERYTHRO-KINETICS IN INFANCY

X. *Red Cell Volume of Newborn Infants in Relation to Gestational Age*

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Knowledge of the circulating red cell volume (RCV) during the foetal life of the human being is of general interest. Furthermore such data contribute to the evaluation of production and destruction of red blood cells during foetal life and early infancy

Brody & Nilsson (4) estimated the circulating haemoglobin mass from plasma volume (PV) measurements with a T 1824 technique in 14 newborn infants of varying intrauterine age (from .35 days to 303 days). They found only a weak relationship (with a non-significant correlation coefficient of 0.50) between total haemoglobin .. and gestational age.

Usher *et al* (11) and Usher & Lind (12) have published a number of data of RCV calculated from PV measurements in newborn infants of recorded gestational age. Taking values of 29 infants from these two studies together there is a good correlation between RCV and gestational age in a gestational age range of 217 to 294 days. However the data are arranged in two groups so that only values from one infant are present in the gestational age range of 245 to 280 days.

In view of this apparent discrepancy and the gap in the data given, and since the earlier studies were performed as PV and not as RCV determinations, the problem was reinvestigated in the present study RCV measurements with a ^{51}Cr dilution method were performed in 27 newborn infants with a gestational age of 222–295 days.

MATERIAL AND METHODS

Twenty-seven infants with gestational age of 222–295 days were studied within 72 hours after birth. Each of five infants (Nos. 1 2, 3 5 and 6) was one of dichorial twins. All infants were normal except for the premature delivery in some f them. They were the progeny of healthy mothers, born after otherwise uncomplicated pregnancies and vaginal deliveries. Their umbilical cords were clamped after cessation f the pulsations, which usually occurred about 5 minutes after birth. Gestational age was calculated from the first day of their mothers' last menstrual period. All infants of a gestational age of less than 260 days were receiving care at the paediatric clinic because of their low birth weight and general prematurity

RCV was determined using ^{51}C -labelled, autologous red blood cells according to method described in detail earlier (2). With this technique, the error of single measurement is $\pm 3\%$ The venous haematocrit was determined by means of an International Microcapillary centrifuge model MB the centrifugation time was 5 minutes. Correction was made for an assumed amount of 1.5% of trapped plasma (6).

RESULTS

The individual values of gestational age, birth weight, age at time of investigation, haematocrit and red cell volume are shown in Table 1

The relationship between RCV and gestational age is shown in Fig. 1 Within the range of gestational age studied, there was a gradual increase of RCV with increasing gestational age. After 230 (± 10) days of intrauterine life the RCV ranged between 45 and 100 ml. Fifty days later at the expected time of delivery these values ranged between 120 and 190 ml. There was thus a daily increase in RCV of approximately 1 7 ml during this interval. The RCV values of the twins of different gestational age do not differ from the corresponding values of the uniparous infants.

The relation between RCV/kg birth weight and gestational age in the infants of the present study

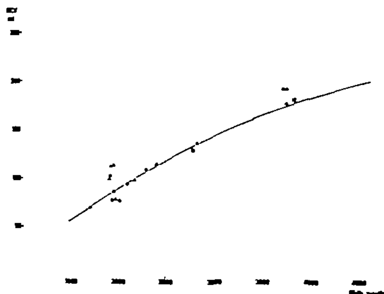


Fig. 3. The correlation between red cell volume and birth weight in the 70 infants shown in Fig. 1. The regression equation is RCV (ml) = −60.27 + 89.52 kg 7.26 kg² (standard error of regression coefficients 2.1 and 1.68).

et al. (11) and Usher & Lind (12) performed their plasma volume measurements using ^{131}I-labelled albumin as a tracer and took the blood samples 5 minutes after injection. As discussed in a previous paper (3) it might be expected that they would have underestimated the RCV when they used the body/venous haematocrit ratio of Mollison *et al.* (10), since these authors took their blood samples 10 minutes after injection. Assuming a disappearance of the tracer of 3% in 5 minutes (2) and using the body/venous haematocrit ratio of 0.91 valid as a correction factor at zero time after injection (?) the RCV values calculated by the present author from the results of Usher *et al.* (11) will be significantly larger than those calculated from the results of Brody & Nilsson (4). However, as pointed out earlier (3), it seems probable that the disappearance of the tracer of Usher *et al.* (11, 12) in 5 minutes was approximately as large as the disappearance of the tracer of Mollison *et al.* (10) in 10 minutes. With this assumption the RCV values calculated by Usher *et al.* (11) and Usher & Lind (1) are correct, and these values are then also in agreement with the results of Brody & Nilsson (4) and of the present study.

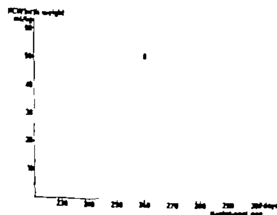


Fig. 4. The relation between red cell volume/kg birth weight and gestational age in 27 infants studied by the present author.

Because of the large variation it is not possible to ascertain whether the relationship between RCV and gestational age is linear or not. The source of this large variation is unlikely to be due to methodological errors in the determination of RCV, since the precision of the method used in this work, as in the studies of Usher *et al.* (11) and Usher & Lind (12), is quite high. Differences in the amount of placental transfusion may contribute to this variation. Another main source of the large variation is most likely the well known large possible error in determining the gestational age from the date given for the first day of the last menstrual period of the mother. This is illustrated by the fact that the relation between RCV and birth weight is closer (Fig. 3) than that between RCV and gestational age. As pointed out earlier, variations occur in rate of foetal maturation. It is not possible, however, to evaluate how

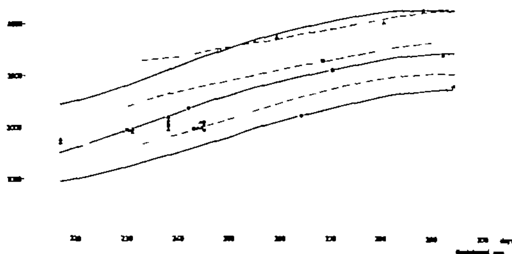


Fig. 2. The relationship between birth weight and gestational age in 27 infants studied by the present author (●), and 43 infants studied by Brody & Nilsson (4) (□) and Usher *et al.* (11 and 12) (Δ). The mean birth weight and 5 and 95 percentile confidence limits after different gestational periods from the work of Hosemann (8) (——) as well as the mean birth weight and 10 and 90 per centiles from the work of Engström & Sterky (5) (– – –) are shown.

riods. This possibility arises from the fact that at a gestational age of less than 260 days infants of low birth weight were selected, since normal infants of a birth weight higher than 2300–2500 g were not attending the clinic for premature infant care. It must be remembered, however, that a sample of infants which is quite representative of all infants born prematurely after different lengths of intrauterine life is not representative of the foetus which remains in utero and is born 280 days after the beginning of the mother's last menstrual period. Some infants are prematurely born as a result of accelerated foetal maturation (8) and this may result in a higher body weight among the group of prematurely born infants in comparison with those who remain in utero for the expected time. It thus seems possible that the infants of the studies discussed in this paper (Brody & Nilsson (4), Usher *et al.* (11, 12) and the present study) are more representative of the foetus remaining in utero than a representative sample of prematurely born infants of the same range of gestational age.

Another point to consider is whether the RCV's of newborn infants of different gestational ages are representative of those of the corresponding foetuses. This will be true if the efficiency of the placental transfusion is unrelated to gestational age. The restrictions discussed above must be borne in mind when evaluating the results.

Brody & Nilsson (4) performed their plasma volume determinations in essentially the same way as did Mollison *et al.* (10) when they estimated the body/venous haematocrit ratio of 0.87 in newborn infants. Assuming that this ratio is also valid for newborn premature infants, fairly accurate estimations of total blood volume, red cell volume and total haemoglobin mass should be possible on the basis of the data of Brody & Nilsson. The RCV values after different lengths of intrauterine life calculated from their results are in agreement with the findings of the present study. The principle reason for the poor and non-significant correlation ($r = 0.50$) between total haemoglobin mass and gestational age found by Brody & Nilsson seems to be that the distribution of gestational ages in their study was skew with only 2 infants of a gestational age less than 265 days.

The two following categories of infants in the works of Usher *et al.* (11) and Usher & Lind (12) were used for comparison with the data of the present work: (*a*) infants with delayed cord clamping and (*b*) truly premature infants. In each infant the mean values of the determinations performed within the first 72 hours were used. Usher

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ENDOGENOUS FORMATION OF CARBON MONOXIDE IN NEWBORN INFANTS

III ABO Incompatibility

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Following the discovery of the ABO blood group system, the effect of the maternal isoagglutinins on the health of the foetus and the newborn infant in ABO heterospecific pregnancies evoked much interest (10). As early as 1923 Ottenberg suggested that maternal isoagglutinins could be responsible for the jaundice frequently observed in newborn infants (15) but before it became possible to separate jaundice caused by Rh isoimmunization, one could not usually demonstrate an untoward effect of ABO incompatibility. In 1944 however Halbrecht reported that in infants with jaundice appearing within 24 hours after birth, ABO incompatibility was found in 95 per cent, if infants with Rh haemolytic disease were excluded (8). This finding has been confirmed by numerous, subsequent investigations. The high frequency of ABO incompatibility far in excess of that found in unselected pregnancies, indicates a connection between the blood group incompatibility and early jaundice. Red cell survival studies in infants with this clinical entity have demonstrated a shortened survival of erythrocytes of the infant's blood group but normal survival of simultaneously administered group O erythrocytes, establishing a haemolytic disease (2, 13). The demonstration of anti-A and/or anti-B with so-called immune characters in the maternal sera and in the circulation of the affected infants is further evidence for an immune haemolytic mechanism, justifying the concept of ABO haemolytic disease. A review of the extensive literature on this subject can be found in Fischer's monograph (6). However such antibodies are common in pregnant women as well as in situations not connected with pregnancy (12), and a pure serological definition of ABO haemolytic disease has not been possible. In mild cases of this condition the jaundice cannot be distinguished from physiological jaundice, and therefore it is difficult to assess the frequency of increased haemolysis due to ABO incompatibility.

In adults with haemolytic diseases the concentration of carboxyhaemoglobin (COHb) in blood has been shown to reflect the degree of haemolysis (5, 20). Increased COHb concentrations have also been found in Rh haemolytic disease of the newborn (7, 14). In order to assess the frequency and extent of increased haemolysis in ABO incompatibility the COHb level was determined in newborn infants with jaundice associated with ABO incompatibility. Furthermore, the COHb concentration in full-term, healthy infants of ABO heterospecific pregnancies was compared with that in infants of ABO homospecific pregnancies. Since increased COHb concentrations have been found in icteric newborn infants without blood group incompatibility (1) the COHb concentrations in such infants and in newborns with icterus and ABO incompatibility were compared.

MATERIAL

In the present investigations only infants of non-smoking mothers were included. Some mothers had preeclampsia, otherwise no diseases of importance were found during pregnancy. No infant was delivered by caesarean section, forceps or vacuum extractor. The infants were studied without regard to the use of nitrous oxide during the delivery (1), but if volatile anaesthetics such as diethyl-

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In ABO heterospecific pregnancies, in contrast to homospecific, the natural isoagglutinins in the maternal serum are antagonists to the foetal ABO blood group. This condition is also called ABO incompatibility.

Table 1 *Criteria for selection and number of cases*

	Main criterion for selection	
	Jaundice	Blood groups in mother and infant
ABO Incompatibility	Group I ($n=62$)	Group II ($=46$)
No incompatibility	Group IV ($=46$)	Group III ($n=61$)

ether and trichlorethylene had been used, no COHb values from the first day of life were included (7). Most infants received 1 mg Vitamin K_4 (menadiolsodiumdiphosphate), none received more than 2.5 mg.

No infant was premature by weight. Infants with signs of asphyxia, espiratory disease cerebral damage or infection were excluded, as well as infants th cephalhaematoma or other haemorrhages.

When Rh incompatibility occurred between infant and mother only infants of non-immunized primiparas, and with negative direct antiglobulin reactions were included.

Four groups of newborns were studied. Criteria for selection and number of cases are shown in Table 1.

Group I. *Sixty-two infants selected because of jaundice associated with ABO incompatibility* Fifty-four of the infants were referred t the department of paediatrics from various hospitals in the region, eight were studied in one maternity hospital in the city All but two mothers belonged to blood group O most of the infants to blood group A (Table 2). "Immune agglutinins could be strated in 3 of 59 maternal sera investigated. A positive direct antiglobulin reaction was found in three infants. In the remaining infants the reaction was negative. Twenty-two of the infants were observed to be jaundiced before 24 hours of age, in further 26 cases jaundice was observed before 48 hours. An increased number of reticulocytes and or spherocytosis were often found, both in infants whose jaundice was observed before 24 hours after birth and in the other infants.

Group II *Forty-six infants of ABO heterospecific pregnancies selected in one maternity hospital without regard to the degree of bilirubinaemia.* None of these infants displayed appreciable early jaundice. All mothers belonged to blood group O 37 of the infants to group

Table 2. *Blood groups in mothers and infants in group I and group II (see text)*

Group I				Group II			
	Mother				Mother		
Infant	O	A	B	Infant	O	A	B
A	52	—	1	A	37	—	—
B	8	—	—	B	9	—	—
AB	—	1	—	AB	—	—	—

A and 9 to group B Immune agglutinins were found in eight of the maternal sera. The direct antiglobulin reaction was negative in all infants.

Group III. *Sixty-one infants of ABO homospecific pregnancies selected without regard t the degree of bilirubinaemia.* These infants were studied during the same period and in the same maternity hospital as the preceding group, but for practical reasons paired sampling was not possible.

Group IV *Forty-six infants with jaundice not caused by Rh isoimmunization or associated ith ABO incompatibility* Thirty-four of the infants were studied in the maternity hospital (group IV *a*), the remaining twelve were referred to the department of paediatrics because of more intense jaundice (group IV *b*) The direct antiglobulin reaction was negative. No systematic attempts were made to exclude rare haemolytic diseases but routine haematological investigations did not indicate the presence of haemolytic disease. Twenty-nine of the infants in this group have been included in a previous report (1).

METHODS

The carbon monoxide content of the blood was deter mined according t Linderholm *et al.* (11) after releasing the CO with sulphuric acid in an extraction chamber and analyzing the extracted gas in Hopcalite CO meter (AB Stillex, Stockholm). The per cent of COHb was calculated from the CO content and the haemoglobin concentration using 1.34 for the CO combining power of haemoglobin. The random error of the method was calculated from duplicate analyzes t be ± 0.05 per cent COHb.

The serum bilirubin concentrations were determined in the department of clinical chemistry at the Children hospital with the method mentioned in the previous report (1). All sera were analyzed in duplicate and the random error of single analysis was calculated to be ± 0.4 mg per 100 ml.

Blood grouping and serological tests were performed at the blood bank of the hospital. Immune agglutinins were demonstrated with the indirect antiglobulin reaction after destroying the natural agglutinins by heating.

Ordinary methods were used for the statistical description of the results (21). The COHb level in group I was compared with that in normal newborns by the Wilcoxon two-sample-rank test. COHb and bilirubin concentrations in group II and group III were compared by Student's *t*-test. The relation between COHb and bilirubin in different groups was compared by analyses of covariance according to Dern (4) or by comparison with the aid of adjusted means. A five per cent significance level as used ($p < 0.05$).

RESULTS

The sixty-two infants selected because of jaundice associated with ABO incompatibility (group I) had significantly higher COHb levels than normal



Fig. 1 COHb level in newborn infants, selected because of significant jaundice associated with ABO incompatibility (group I). Four infants are represented by two values (symbols connected by broken lines). Mean values for healthy non-icteric newborns are connected by solid line, ± standard deviations by broken lines.

non-icteric newborns (7) (Fig. 1 Table 3). In 48 of the 62 infants the COHb concentration exceeded the mean level for normal newborns by more than two standard deviations (Fig. 1)

When newborn infants selected without regard to the degree of bilirubinaemia were investigated, slightly higher COHb level was found in infants of ABO heterospecific pregnancies (group II) than in infants of ABO homospecific pregnancies (group III) (Table 3). On days 4–6 the difference between the groups was statistically significant. The bilirubin concentration in the two groups did not differ significantly (Table 3).

The relationship between COHb and bilirubin in group I and group II on different days is shown in Fig. 2. The small number of valuable data does not permit an analysis of this relationship on the first day of life. A significant correlation between COHb and bilirubin was found on days 3 and 4–6 in group I and on days 2 and 3 in group II (Table 4). The COHb/bilirubin relation in the groups was compared, for day 2 with the aid of adjusted means, and for day 3 by analysis of covariance. No significant difference was found. Thus, during the first days of life the COHb/bilirubin relation in infants with ABO incompatibility whether selected because of jaundice or selected irrespective of the degree of bilirubinaemia, can be regarded as identical (Fig. 2).

In full-term newborn infants with jaundice not caused by blood group incompatibility or other known haemolytic disease, we have earlier reported increased COHb concentrations significantly correlated to the bilirubin level (1). In an extended material (group IV) the same relationship between COHb and bilirubin as reported previously was found after the third day of life (Fig. 3 Table 4). If only the results from the infants studied in the maternity hospital (group IV *a*) were analyzed, a correlation was still found (Table 4) Comparison with the aid of adjusted means did not show any significant difference be-

Table 3 *COHb and bilirubin concentrations in groups I–IV on different days*

COHb concentrations in normal non-icteric newborns are given for comparison

		Day 1			Day 2			Day 3			Day 4 and later		
			Mean	s.D.		Mean	s.D.		Mean			Mean	s.D.
Normal newborns	COHb	1	1 04	0.16	12	0 9	0.13	14	0 93	0 11	28	0 77	0.1
Group I	COHb	3	1 60		13	1 73	0 38	21	1 51	0 30	28	1.23	0.28
	Bilirubin	3	12.0		13	21 9	4 5	21	23 6	5 8	28	23 4	6.4
Group II	COHb	5	1 13		15	0 99	0 17	21	1 02	0 20	28	0 98	0.19
	Bilirubin	5	5 0		15	6 4	3 2	21	7 8	4 8	28	8.7	5.5
Group III	COHb	13	1.08	0 28	24	0 96	0 17	30	0 99	0 15	35	0 90	0 13
	Bilirubin	13	4 9	1 9	24	5 8	2.5	30	7 4	3 6	35	9 0	4 7
Group IV	COHb		—			1 05		19	0 98	0.19	21	0 90	0 22
	Bilirubin		—		2	9 3		19	13 6	2.6	1	14 2	5
Group IV *b*	COHb		—			—			—		12	1 4	0.12
	Bilirubin		—			—			—		1	29 1	3 2

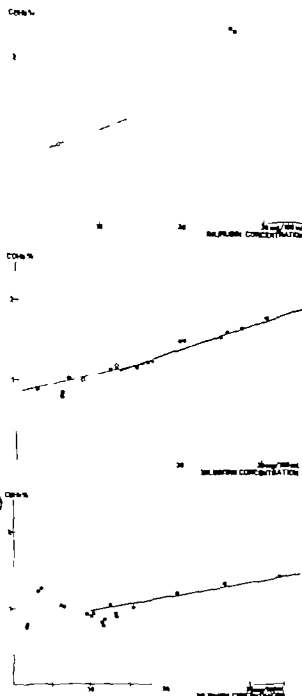


Fig 2 Relation between COHb and bilirubin on different days in infants with ABO incompatibility selected because of significant jaundice (group I, filled symbols), or without regard to the degree of bilirubinaemia (group II, open symbols). Day 2 (upper figure) A significant correlation is found in group II ($\hat{y} = 0.038x + 0.75$, $s^2_{y \cdot x} = 0.017$ broken line). Day 3 (middle figure) A significant correlation is found in group I ($\hat{y} = 0.035 \;\; + 0.67$, $s^2_{y \cdot x} = 0.053$, solid line) and in group II ($\hat{y} = 0.022x + 0.85$, $s^2_{y \cdot x} = 0.029$ broken line). Days 4–6 (lower figure) A significant correlation is found in group I ($\hat{y} = 0.018x + 0.80$, $s^2 \;\; = 0.070$, solid line)

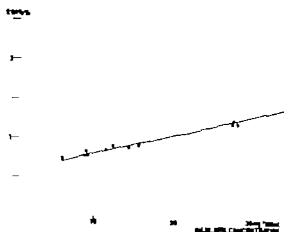


Fig 3 Relation between COHb and bilirubin after the third day of life in infants selected because of jaundice without known haemolytic disease (group IV). Infants studied in the maternity hospital (group IV) represented by open symbols, infants studied in the department of paediatrics (group IV *b*) by filled symbols. A significant correlation is found in group IV ($\hat{y} = 0.023x + 0.58$, $s^2_{y \cdot x} = 0.027$ solid line) and in its subgroup IV ($\hat{y} = 0.026x + 0.53$ $s^2_{y \cdot x} = 0.031$)

tween the COHb/bilirubin relation in this group and in the infants with higher bilirubin levels studied in the department of paediatrics (group IV *b*) On day 3 no correlation was found between COHb and bilirubin in group IV In infants of ABO homospecific pregnancies selected irrespective of the degree of bilirubinaemia (group III) a correlation between the two variables was found on day 1 but not later (Table 4)

Table 4. *Relation between COHb and bilirubin given as correlation coefficients* (r)

	Day 1	Day 2	Day 3	Day 4 and later
Group I	—	−0.07 (−13)	−0.67* (−21)	0.41 (28)
Group II	—	−0.69* (−15)	−0.54* (− 1)	0.17 (−28)
Group III	−0.56* (n = 13)	−0.20 (n = 24)	−0.20 (30)	0.03 (n 35)
Group IV	—	—	−0.21 (−19)	−0.78* (33)
Group IV	—	—	−0.[illegible] (19)	0.61 (− 1)
Group IV *b*	—	—	—	− 0.19 (−12)

The hypothesis that ρ equals zero is rejected at the five per cent level.

The linear regression of bilirubin upon COHb after the third day in group I and in group IV was compared by analysis of covariance, which demonstrated that the regression coefficients did not differ but that the vertical distance between the regression lines differed significantly from zero ($t = ..03..$). This implies that for every bilirubin value within the range observed there was a difference between the mean COHb in group I and in group IV The difference, 0.12 per cent COHb, was of the same magnitude as the difference between the mean COHb concentration in group II and group III at this age (Table 3).

DISCUSSION

In ABO incompatibility a haemolytic disease is usually considered when the infant presents appreciable, early jaundice or anaemia. In such cases red cell survival studies in the infant's circulation have shown a shortened life span of transfused homologous erythrocytes (2, 13). The frequent demonstration of anti-A and/or anti-B with so-called "immune properties, such as resistance to heating, resistance to neutralization with soluble blood group substances, and enhanced agglutination in protein medium, has indicated an immune haemolytic disease. During recent years these "immune properties have been shown to depend on the presence of anti-A and/or anti-B belonging to the 7S globulins, capable of passing the placental barrier (9 16, 17) Antibodies with these serological and immunochemical characters are, however common in persons belonging to blood group O (9 12, 16), and consequently there are possibilities for a haemolytic disease in many infants of ABO heterospecific pregnancies. Although most of these infants do not show any obvious clinical symptoms, some investigations have indicated that moderately increased haemolysis due to ABO incompatibility is common during the newborn period (18 19 22).

In the present investigation no attempt was made in advance to define an ABO haemolytic disease clinically or serologically The two groups of infants, incompatible with their mothers with respect to the ABO blood groups, were chosen to represent the tw forms of this condition met by the pediatrician responsible for the care of newborns. Group I consisted of infants with jaundice, in whom the risk of bilirubin encephalopathy and the indication for exchange transfusion had to be evaluated. Several of these infants would fulfill rigorous clinical criteria for the diagnosis of ABO haemolytic disease demonstrating early and pronounced jaundice, increased number of reticulocytes, and spherocytosis. Among the remaining infants in this group some had symptoms suggesting a haemolytic disease but in others the clinical picture was indistinguishable from physiological jaundice. Group II comprised healthy full-term infants of ABO heterospecific pregnancies as they are seen in the maternity wards. In the statistical analysis of the results, group II was regarded as a random sample of a population consisting of infants of ABO heterospecific pregnancies, fulfilling the criteria set in the description of the material. These criteria aimed at eliminating, as far as possible, exogenous and irrelevant endogenous factors influencing the COHb level. To the best of our knowledge, no other factor than ABO incompatibility selectively could have influenced the COHb level or the COHb/bilirubin relation in group II.

Rh(D)-isoimmunization was excluded in both groups, but the maternal sera were not screened for other intragroup antibodies. The direct antiglobulin reaction was, however negative in all but three infants who showed a weak, positive reaction. This finding is not consistent with isoimmunization, except within the ABO blood group system. Other congenital haemolytic diseases were not looked for systematically but are rare in this part of Sweden. Therefore it can be assumed that the signs of increased erythrocyte destruction found in the above-mentioned groups can be attributed to the ABO incompatibility

The influence of different factors on the COHb level in blood has been discussed in detail by Coburn *et al* (3), who stressed the importance of exogenous CO and of the alveolar ventilation. As in our previous investigations (1 7) the effect of exogenous CO and of variations in pulmonary function have been reduced through selection of the material. The COHb concentrations found i the present material are therefore assumed to depend mainly on the degree of haemolysis.

In newborn infants with jaundice associated with ABO incompatibility (group I) markedly increased COHb concentrations were found (Fig.

1 Table 3) During the first three days, mainly infants with clinical signs suggesting haemolytic disease were studied In these infants the COHb concentrations were of the same magnitude as those found in infants with moderate to severe Rh haemolytic disease (7) ABO haemolytic disease is generally regarded as a mild haemolytic disease, with bilirubin encephalopathy as a serious menace but practically never anaemia or hydrops. In agreement with this opinion none of the in fants in the present material was anaemic. On the other hand anaemia was often found in Rh haemolytic disease with the corresponding COHb level (7) This difference between the two dis eases might be explained by shorter duration of haemolysis, or by greater regenerative capacity of blood forming tissues in ABO haemolytic dis ease.

Several of the infants in group I investigated after the first three days of life did not display unequivocal signs of haemolytic disease. At this age a lower COHb level was found than previously although still significantly increased in comparison with that in normal newborns (Fig. 1 Table 3). In these infants the increased COHb level need not necessarily be attributed to the ABO incompatibility since COHb concentrations in the same range were often found in jaundiced infants without blood group incompatibility (group IV) (Fig. 3 Table 3) The COHb/bilirubin relation in the two groups differed, however significantly (Figs. 2 and 3) and the difference indicates that also at this age increased haemolysis was of greater importance in the pathogenesis of the bilirubinaemia in group I than in group IV We conclude that this difference should be attributed to the ABO incompatibility

The slight but significant difference between the COHb concentrations in full-term healthy infants of ABO heterospecific pregnancies (group II) and ABO homospecific pregnancies (group III) is consistent with the small difference in bilirubin concentrations found in similar materials published earlier (18, 19). In the present investigation the bilirubin concentration in the two groups did not differ significantly The results indicate that the COHb level is a more sensitive indicator of haemolysis than the bilirubin con centration.

During the first days of life there was a significant correlation between COHb and bilirubin in group II, and the same relation between COHb and bilirubin was found in group I as in group II (Fig. 2, Table 4). This finding is of great theoretical interest, as it indicates that the pathogenesis of the bilirubinaemia in the two groups is identical, only differing in degree. After the third day a significant correlation between COHb and bilirubin was no longer found in group II. Evidently at this age factors other than haemolysis, such as hepatic maturity influence the bilirubin level to a greater extent in this group. On the other hand the significant correlation between the two variables was still found in group I, indicating a more dominating influence of haemolysis in that group.

The increased COHb level and the significant correlation between COHb and bilirubin found in icteric infants without blood group incompatibility have been discussed in an earlier report (1). We believe that it denotes an increased rate of haemoglobin catabolism in these infants. In the extended material presented here (group IV), a significant linear regression of bilirubin upon COHb was also found, when the analysis only included results from the infants studied in one maternity hospital (Fig. 3 Table 4). It is not likely that in this limited population the correlation was influenced by the inclusion of unrecognized cases with haemolytic disease. The results therefore strengthens our opinion that increased haemoglobin catabolism usually contributes to the physiological jaundice of the newborn

In infants of ABO heterospecific pregnancies increased haemolysis due to the blood group incompatibility is thus not restricted to infants with unequivocal signs of haemolytic disease but contributes to the hyperbilirubinaemia in most icteric infants. Furthermore, slightly increased haemolysis is common in infants of unselected ABO heterospecific pregnancies, although not of a magnitude to be of clinical importance. These findings are consistent with the results of earlier serological and immunochemical investigations, which have shown that anti-A and anti-B, capable of passing the placental barrier are common in mothers with blood group O (9 12, 16).

SUMMARY

The carboxyhaemoglobin (COHb) concentration in blood, which is assumed to reflect the degree of haemoglobin catabolism, was determined in 6. newborn infants selected because of significant jaundice associated with ABO incompatibility and in 46 infants with ABO incompatibility selected without regard to the degree of bilirubinaemia. Furthermore, the COHb level was determined in 46 icteric infants without haemolytic disease, and in 61 infants of ABO homospecific pregnancies, selected without regard to their bilirubin level.

In infants with ABO incompatibility increased COHb concentrations were found not only in infants with unequivocal signs of haemolytic disease but also in infants with a clinical picture indistinguishable from physiological jaundice. During the first days the relation between COHb and bilirubin in infants with ABO incompatibility was the same, whether the infants were selected because of significant jaundice or irrespective of the degree of bilirubinaemia. This indicates a common pathogenesis of the bilirubinaemia in the two groups.

The COHb and bilirubin levels in infants of ABO heterospecific pregnancies and in infants of ABO homospecific pregnancies, selected without regard to the degree of bilirubinaemia, were compared. It is concluded that the COHb level probably is a more sensitive indicator of haemolysis than the bilirubin concentration.

Increased COHb concentrations were also found in icteric infants without blood group incompatibility but the relation between COHb and bilirubin indicates that in ABO incompatibility increased haemolysis is of greater importance in the pathogenesis of the jaundice.

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CASE REPORT

CONGENITAL FIBRINOGEN DEFICIENCY

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Congenital afibrinogenemia is a rare condition, clinically and hematologically well characterized, affecting either sex. According to a recent review of the literature (6) only 56 cases have been reported from various countries of the world since the first report in 1920 (22) The disorder has so far not been described in persons of Greek descent. Clinically the disease is characterized by a hemorrhagic syndrome (5 29), the principal features are: onset of bleeding early in life, frequently by umbilical hemorrhage; bleeding from cuts or other slight injuries subcutaneous hemorrhages; excessive bleeding with the eruption and loss of the deciduous teeth; absence of hemarthroses and of spontaneous hemorrhages; mildness of the bleeding tendency as compared with that of hemophilia. The fibrinogen deficiency is thought to be related to a specific failure of its synthesis (5 12). Characteristic laboratory findings in all reported cases are the complete absence of blood clotting and the almost total lack of fibrinogen (5 1).

The purpose of the present report is to describe a mild *form of* congenital fibrinogen deficiency with a less severe degree of this defect in 9-year-old girl of Greek origin, whose parents are affected by the same disorder in latent form.

CASE REPORT

The patient, D. H., girl born March 15 1957 as the second of two siblings. The pedigree is illustrated in Fig. 1. The patient' father and her maternal grandmother are first cousins. The parents, both of Greek origin, are in good health and had no history of bleeding. No cases of known hemorrhagic diseases or bleeding tendency had been recorded among their near relatives. The mother had two uncomplicated pregnancies. Her first pregnancy in 1954, resulted in the delivery of normal, well developed male, who is now alive and healthy He never had any bleeding manifestations. The patient is the product of her second pregnancy There is no history of illness, drug ingestion or exposure to X-ray during this pregnancy

The patient was born at term after an uneventful normal delivery At birth she was well developed, with eight of 3300 g and length of 50 cm. She was referred for the first time to the Pediatric Clinic of Aristotelian University Thessaloniki, on March 19 1957 at the age of 5 days, because of persistent umbilical bleeding and severe posthemorrhagic anemia (Table 1). The umbilical bleeding followed the dropping of the umbilical cord. The general condition, on admission, was satisfactory The baby weighed 3100 g, and was slightly icteric. Hemoglobin 3.1 g/100 ml; red cells 1 44 $10^6/\mu l$; reticulocytes 3.8% white blood count and differential normal, platelets 220,000/μl; bleeding and clotting times normal. The peripheral blood smears revealed moderate polychromasia and 5 nucleated red cells per 100 white cells The blood groups of infant and mother were both O Rh positive, direct Coombs test negative. The infant was transfused immediately after admission and twelve hours later with 50 ml and 60 ml of fresh blood respectively The bleeding ceased immediately after the first transfusion. On the next day March 20, the hemoglobin as 7 2 g/100 ml and the red cells 2.6 $10^6/\mu l$. The infant was discharged in good condition, 48 hours after her admission. At the age of two years, on April 30, 1959 the patient was readmitted to the same clinic, presenting small cut of the scalp in the right frontal area, about 3.5 cm long, bleeding for six days, with an extensive hematoma of the soft tissues of the whole frontal region and severe posthemorrhagic anemia. On admission the child was very pale, otherwise in good general condition. Hemoglobin 8.2 g/100 ml; red cells 2 5 $10^6/\mu l$, whole clotting time 10 min, bleeding time 3 min. 5 sec. She was transfused with 150 ml of fresh blood, after which bleeding ceased immediately

Table 1 *Clinical history of the patient's hemorrhagic syndrome*

Hemorrhagic manifestations	Age at the event of H. M	Hemoglobin g/100 ml	Treatment
Severe with post-hemorrhagic anemia			
Umbilical bleeding	5 days	3.1	Blood transfusion
Bleeding from cut with extensive regional hematoma	2 years	8.2	Blood transfusion
Extensive hematoma of the whole scalp	2 2/12 years	7 1	Blood transfusion
Mild			
Bleeding after shedding of the primary teeth	5½–6½ years	Normal	Nothing
Ecchymoses-Hematomas after slight trauma	From the 1st year up to day	Normal	Nothing

About two months later on June 12, 1959 she was readmitted to the same hospital because of very extensive hematoma of the whole scalp, without external blood loss, which occurred shortly after a mild stroke on the head.

On admission she was obviously anemic with diffuse bulging deformity of the skull, and a hematoma of the left elbow and hip. The blood examination showed: hemoglobin: 7 1 g/100 ml; red cells 2.4 $10^6/\mu l$; platelets 240,000/μl, white blood count and differential, bleeding and clotting times normal. The patient received 200 ml of fresh blood and the next day was taken home again.

At the age of 5 / years, few hours after the ding of the first deciduous tooth she developed but persistent bleeding which ceased spontaneously within 4 days. The same trouble also occurred after the shedding of the next two teeth. The child had never presented petechiae, epistaxis or other spontaneous hemorrhages from mucous membranes or haemarthrosis. The hemostasis after common cutaneous cuts was normal or was obtained easily On the contrary even slight trauma used t result in ecchymoses or subcutaneous hematomas of varying size especially in the extremities.

At the age of 6 years, on July 20, 1963 she was referred to the Department of Pediatrics, University of Freiburg, for further investigation.

Physical Examination

The patient is an intelligent well developed rather obese girl. She presented a hematoma approximately 9 10 cm over the left hip and several smaller hematomas on forearms and legs. Otherwise she was in good health. Petechiae or hemorrhages from the mucous membranes are not oserved. Nothing abnormal was detected at the cardiovascular and respiratory systems. The liver and spleen were not palpable.

METHODS

The plasma fibrinogen was determined by measuring the nitrogen content of the test clot by means of the Kjeldahl method (28). In the patient's brother the turbidimetric method was used (27).

For determination of factors VIII, IX, and platelet factor 3 the thromboplastin generation test was used (4).

The factor X (Stuart-Prower factor) was determined using the Russel's viper venom test (Stypven time with bovine plasma). Normal range: 70–100%

Factor V was measured by the one stage prothrombin test using factor V deficient plasma (stored human plasma). Normal range 70–100%

For determination of factor VII-complex the one stage prothrombin test with factor VII deficient plasma was used.

In the prothrombin test $BaSO_4$-absorbed bovine plasma and human serum as source of factors V and VII is added to the system. Normal range: 70–100%

The antithrombin was measured by determination of the clotting time in the presence of a certain amount of purified thrombin, using "Testthrombin Behringwerke Normal range: 20–28 sec.

In the prothrombin consumption test the one stage prothrombin time in patient serum after 4 hours was determined Normal range: 0–6%

The fibrinolytic activity was measured by the method described above for the determination of fibrinogen, after incubation of the clot for 24 hours at 37°C in relation to the original fibrinogen level as well as by measurement of the clot redissolution time (21).

In the heparin tolerance test the recalcification time of citrated whole blood in the presence of small amounts of heparin (0.2 U/ml) was determined. Normal range: 135–165 sec.

LABORATORY DATA

The blood examination showed. hemoglobin 13 7 g/100 ml, red blood cells $4.36 \times 10^6/\mu l$, white

Reagens zur Factor VII-Bestimmung "Roche Deutsche Hoffmann La Roche A.-G., Grenzach/Baden.

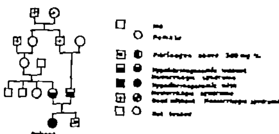


Fig. 1 Fibrinogen deficiency—pedigree of the affected family

blood cells 5500/µl (neutrophils 6 % lymphocytes 35 % eosinophils 1 % monocytes 2 %), platelets 164–228,000 µl, reticulocytes in normal number red cell morphology normal hemoglobin A_2 2.71 % hemoglobin F 0.25 % The direct Coombs test as well as the platelet antibody test were negative. The cold agglutination titer was 9

Electrophoresis of serum proteins showed. Albumin 67 % globulins, $Alpha_1 = 3$ % $Alpha_2 = 8.5$ % Beta = 1 % Gamma = 9.5 % Serum electrolytes. Sodium 136 mEq/l, potassium 3 9 mEq/l, calcium 5 7 mEq/l, chloride 108 mEq/l, inorganic phosphorus .4 mEq/l, bicarbonate 26 mEq/l. Serum alkaline phosphatase 9 Bodansky units. Serum bilirubin 0.17 mg/100 SGOT 16 units, SGPT 18 units. Thymol turbidity test normal. The protein bound iodine w s 5–6 µg/100 ml. Urinary steroids. 17-hydroxycorticosteroids 8 8 mg/.4 h, 17 ketosteroids 1 7 mg/24 h. The urine was normal. The Sabin-Feldmann dye test showed a titer of 1/.56. Syphilis serology toxoplasmosis and listeriosis CBR were negative The antistreptolysin—O titer was normal.

Coagulation and hemostatic function studies The patient's blood presented a marked diminution of the fibrinogen level (66–91 mg/100 ml), while the whole blood clotting time was normal. With the exception of a low value in the prothrombin assay normal values were obtained in all the other coagulation tests (Table 2). The thrombolastogram demonstrated a marked diminution of the maximum amplitude, whereas the reaction time (*r*) and clot formation time (*k*) were within the normal limits (Fig. 2). The same diminution of plasma fibrinogen, although in a smaller degree, was found in both the patient's parents. In the father the fibrinogen was 99 mg/100 ml and in the mother 117 mg/100 ml (Table .)

Table 2. *Data of blood coagulation and hemostatic function*

Test	Normal range	Patient, H D	Father, H. D.	Mother, H. E.	Brother
Heparin tolerance test (sec)	135–165	110	145	135	—
Quick test (one stage prothrombin time) (.)	70–100	86	96	99	98
Prothrombin (%)	70–100	49	62	100	—
Factor VII-Complex ( )	70–100	83	100	100	—
Factor V (%)	70–100	96	100	100	—
Prothrombin consumption (.)	0–6	1	2	5	—
Factor VIII (%)	60–100	100	—	—	—
Factor IX (%)	60–100	100	—	—	—
Factor X (%)	70–100	100	—	—	—
Thrombelastography (min)	12	12	—	—	—
k (min)	6	10	—	—	—
ma	90–150	47	—	—	—
Fibrinogen (mg/100 ml)	200–400	66–91	99	117	350
Fibrinolysis (mg/100 ml)		63–78	61	82	—
Clot redissolution time (hrs)	72	72	72	72	72
Calcium clotting time (sec)	90–100	75	75	75	89
Antithrombin (sec)	70–78	27	—	—	—
Platelet factor 3 (sec)	60–100	100	—	—	—
Bleeding time (sec)	240	111	108	110	95
Whole blood clotting time (Lee-White) (min)	6–12	8 ½	7 ½	7 ½	6 ½
Clot retraction		Normal	Normal	Normal	Normal
Rumpel–Leede Test		Negativ	Negative	Negative	Negative

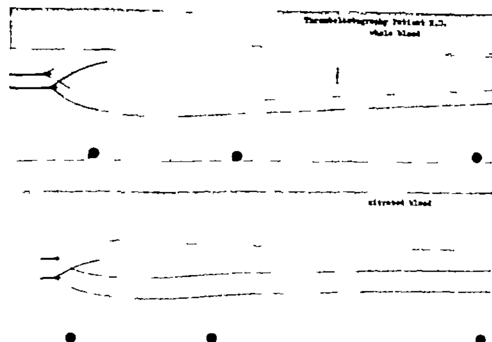


Fig Thrombo-elastogram of the patient with congenital fibrinogen deficiency

Their bleeding and clotting times were normal *The results of the other tests were also normal* The patient's low prothrombin value must not be explained by the low fibrinogen content, its cause has to remain uncertain. Bleeding and clotting as well as plasma fibrinogen in the patient's ~ were normal (Table 2).

CLINICAL COURSE

After discharge from the Clinic, on July 30, 1963 and up to date the patient continues to present ecchymoses and subcutaneous hematomas of various size, mostly on the arms and legs. However no other hemorrhagic manifestations appeared during the abov time and the shedding of the other deciduous teeth was not accompanied by bleeding. Likewise, the exanthematous diseases (measles, varicella, rubella, scarlet fever) by which she was affected during the school period had a normal course and their exanthema did not become hemorrhagic.

DISCUSSION

The hemorrhagic syndrome described presents the principal features of congenital afibrinogenemia, hemorrhagic tendency with onset of bleeding early in life, absence of hemarthrosis and of spontaneous hemorrhages. However it *differs from* it as to the severity of the hemorrhagic syndrome and mainly as to the extent of the biological disturbance. It is known that patients with afibrinogenemia are much less severely affected by bleeding complications than the average hemophiliac (5 16, 18, 29). Nevertheless the prognosis of this condition is rather serious and in the past when treatement was unsatisfactory it was not uncommon for these children to die early Thus, among 24 cases reported up to 1954 9 died, most during infancy (17)

It is true that our patient presented serious manifestations until the age of 2 1/2 years, similar to those observed in congenital afibrinogenemia. Since then and up to date, for 7 years, no other dangerous hemorrhagic episodes took place; especially during the last 3 years the bleeding tendency has been manifested simply by ecchymoses or subcutaneous hematomas, usually of moderate size.

In congenital afibrinogenemia, although the bleeding is also most severe during infancy *it* continues to be serious even in childhood and early adult age (3 10 ̲0, ̲5 22).

The almost total lack of fibrinogen and the complete absence of clotting are the principal laboratory findings in congenital afibrinogenemia (5 21). Gitlin & Borger (12), using the more sensitive immunochemical method have shown that there is not more than 1 mg/100 ml of fibrinogen present in the plasma of these patients. All the other known clotting factors are

normal (11 13). A transient and moderate thrombocytopenia has been reported in a few cases (13 18) but its presence has been doubted (21).

In our case all the known clotting factors other than fibrinogen as well as the thromboplastin generation were normal. However in contrast to the classical afibrinogenemia, the lack of fibrinogen is partial and is accompanied by normal clotting time.

A moderate decrease of fibrinogen levels was found also in some of the parents and in many near relatives of known cases of congenital afibrinogenemia, but none of them developed clinical symptoms of bleeding (5 9 15 16, 18) as did our patient.

Reduced amount of plasma fibrinogen was also a common laboratory finding in 4 cases of a hemorrhagic syndrome reported by Risak (24) under the term constitutional fibrinopenia and in one similar case of Allibone & Baar (1)

These cases constitute a not well-defined condition (5) probably different from congenital fibrinogen deficiency

The four cases of Risak (24) refer to adult women in whom the plasma fibrinogen was greatly reduced (18–20 mg/100 ml) and an excessive bruising, bleeding of the gums and purpura were present. The bleeding time and the clotting time were normal or moderately prolonged. In 3 cases there was also a moderate thrombocytopenia. The haemorrhagic manifestations in all these cases did not appear as early as in congenital afibrinogenemia. The fifth case (1) concerns a female new-born infant with some blood oozing from the umbilicus, which died on the fifteenth day of life from cerebral hemorrhage. The fibrinogen in this case was 110 mg/100 ml and the clotting time over 4 hours. The severe liver changes found in autopsy the discrepancy between the degree of fibrinopenia and the prolongation of clotting time, the intense thrombocytopenia and the atypical hemorrhagic manifestations (spontaneous bruises on the cheeks, endocranial hemorrhage) distinguish this case from the congenital fibrinogen deficiency

Therefore we believe that the above 5 cases of constitutional fibrinopenia cannot be included in the same disease entity

Our case presents the characteristics of congenital fibrinogen deficiency but in a less severe degree, so it can be considered to be its mild form.

The amelioration of the bleeding tendency in our patient as she was growing-up should also be emphasized. An analogous evolution of the bleeding is also observed in the classical afibrinogenemia as well as in hemophilia (29), although the clotting defect remains quantitatively unchanged throughout life. This may be ascribed to the delicate structure of the vessels in the baby and to greater resistance to injury as the child grows up. Also part of the improvement can be ascribed to the more vigorous anatomic structure of the older child as well as to the fact that the patient has learned to be cautious.

The genetic problem of our familial cases of fibrinogen deficiency as well as their relation by genetic point of view with the cases of congenital afibrinogenemia presents a point of interest. Certainly a definite answer to these problems is not possible with the available data and without other similar observations. However some hypotheses can be made. Congenital afibrinogenemia appears to be transmitted by a non-sex-linked recessive gene (4 5 18 25 29). According to Schönholzer (25) the heterozygotes have only a latent fibrinogenopenia while the homozygotes have true afibrinogenemia.

I our family since the defect is transmitted according to the recessive mode of inheritance by the same abnormal gene, it should be accepted that the patient's parents are heterozygotes and the patient herself homozygote.

This mode if inheritance is suggested by the following genetic findings: (1) The consanguinity of the parents of the affected girl, (2) the constatation of a minor and latent form of the same defect in both of these.

To explain the mildness of the disease in our patient one may postulate that the gene which governs the fibrinogen formation can either vary quantitatively or is an allelic mutant occuring at the same locus as the normal gene and the classical afibrinogenemic gene.

Recently an inherited abnormality of fibrinogen resulting in an asymptomatic clotting defect or a mild hemorrhagic diathesis was described (2). This defect is controlled by an abnormal dominant gene and represents dysfibrinogenemia rather than hypofibrinogenemia (2, 19).

Certainly in our cases the possibility of the

presence of abnormal fibrinogen was not investigated, because at the time our cases were studied, we had no knowledge of the above cited fibrinogen abnormalities.

However against this possibility are the different symptomatology of the defect in our cases as compared to the cases of dysfibrinogenemia, and mainly the dominant mode of inheritance and the qualitative rather than quantitative defect in the latter cases.

SUMMARY

A mild form of congenital fibrinogen deficiency is reported in a 9 year-old girl under our observation from birth. She had from early life a history of hemorrhagic syndrome with the principal features of congenital fibrinogen deficiency but with normal clotting time and partial fibrinogen deficiency The patient's parents, related by blood, both of Greek origin, are also affected by the same disorder in a latent form.

The mildness of the hemorrhagic diathesis, especially after infancy and the better prognosis as compared to the classical afibrinogenemia is emphasized.

The genetic problems of these familial cases of fibrinogen deficiency and their relation by genetic point of view with the cases of classical afibrinogenemia are discussed.

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CASE REPORT

ACUTE CEREBELLAR ATAXIA ASSOCIATED WITH HERPES SIMPLEX VIRUS INFECTION

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Acute cerebellar ataxia is a syndrome which has been drawing increasing attention (2, 3 4 8, 9 12). The etiology of acute cerebellar ataxia seems to be heterogenous (varicellae, rubeola, morbilli, polio, pertussis, parotitis, mononucleosis, influenza, ECHO intoxications (2, 3 4 8, 9) but has been properly established through laboratory evidence only in cases of Polio type 1 ECHO types 9 and 6, Coxackie group A, type 2, Coxackie group B, types 3 and 4 and Influenza A and B (2, 3 11).

Hitherto, Herpes Simplex virus has not been recognized as the causative agent of acute cerebellar ataxia. This report deals with the clinical and virological findings in one patient with Herpes Simplex virus infection and acute cerebellar ataxia.

CASE HISTORY

B. L., boy born March 4, 1957 was admitted to the hospital in 1965 at the age of 8 / years because of neurological signs after febrile illness.

Birth and developmental history were normal. He had been vaccinated against TB, tetanus-diphteria-pertussis, and polio (3 injections). At the age of 2 years he fell 1 meter from a bench and was unconscious for about two minutes. One month later he slipped in the bathroom (no unconsciousness) and after that he could not use his left leg for about one week. Thereafter he got slight paresis in his right arm and right leg, where sometimes tremor was seen and the muscular tonus was increased. The EEG and X-ray of the skull were normal. In the following years the difference between right and left side diminished and was seen only when the patient was tired or upset.

On August 22, 1965 he (and his father brother and sister) aquired an upper respiratory infection, fever up to 40° C, headache, vesicles inside the mouth and conjunctivitis. Three days later the fever subsided but the boy experienced vertigo and vomiting when trying to rise from his bed or even altering the position of his head. On admission, August 29 he was tired but totally conscious, unable to stand or sit up because of nausea and vomiting. He showed increased tonus of his masseter muscle and dysarthria. Jerky eye movements were observed. Finger-to-nose and heel-to-knee test revealed dysmetria. He had normal sensibility corneal reflexes, muscle tendon reflexes; no paresis and no nuchal rigidity. The tonsils were slightly reddened (the culture demonstrated beta hemolytic streptococci). The E. S. R. was 58 to 14 mm. The white blood cell count was initially slightly elevated, but in two days sank to normal level. The differential count was normal. A lumbar tap was normal. The EEG (which had been normal in 1961) was pathological with slowing bilaterally and decreased voltage on the left side. Echo-encephalography X-ray of the skull and tomography of the porus acusticus internus appeared normal. Aside from the pathological findings reported, neurological, otological, ophthalmological (except for blepharoconjunctivitis) and ordinary laboratory examination showed conditions within normal limits.

On September 1 10 days after falling ill, the vertigo diminished and he could sit up one day later he still showed severe ataxia, but was able to take a few staggering steps. 15 days after onset he could walk without trouble; his jerky eye movements had disappeared and he had no tremor. Only Romberg test was positive (falling right). When checked 4 months later increased irritability and dyslexia were noticed. At the last check up in February 1967 only dyslexia and his right-side-weakness were seen. EEG taken 1 month and 1 years after onset of the illness were normal.

VIROLOGICAL EXAMINATION

An attempt to isolate Coxackie, Polio, ECHO Adeno and Herpes Simplex viruses from faeces by means of inoculation into newborn mice was made but with negative results. The complement fixing antibody titer to Herpes Simplex was determined at the regional diagnostic laboratory

	Days after onset	Umeå	Stockholm
Acute serum	8	64	160
Conv serum			
I	19	128	320
II	33	—	160
III	163	16	—
IV	534	4	40

(Umeå) and the National Bacteriological Laboratory (Stockholm).

Complement fixing antibody titers to Parotitis, Adeno-viruses, RSSE, Polio types 1 2 and 3 and Influenza A and B all turned out negative.

DISCUSSION

This patient obviously suffered from acute cerebellar ataxia, the diagnosis being based on acute vertigo preceded by fever vomiting without signs of meningitis, severe ataxia, jerky eye movements, dysarthria, EEG abnormality of short duration, normal lumbar tap and hasty disappearance of the ataxia. The clinical pattern of the disease (upper respiratory infection, conjunctivitis, high-fever vesicles inside the mouth before the ataxia developed) is similar to that seen in Herpes Simplex infection.

The only way to establish a virological diagnosis in diseases of deeply situated organs, such the central nervous system, is to isolate the us itself and to demonstrate an immunological response (1 5 6). In this case we were not able to prove the diagnosis by isolation of the virus (and the neutralization test has not been performed). However the complement-fixing antibody titer to Herpes Simplex estimated in two different laboratories demonstrated, though at different levels, the same trends: elevation followed by diminution. These findings together with the clinical picture makes the diagnosis of Herpes Simplex infection most likely

Most virus infections in their early stages are looked upon as being generalized throughout the body due to viraemia (7) and it is thus conceivable that the Herpes Simplex virus could cause cerebellar symptoms to arise though, earlier this virus has not been known to cause acute cerebellar ataxia.

The connection between acute cerebellar ataxia and Herpes Simplex virus infection in this patient makes it plausible to assume, that the acute cerebellar ataxia was caused by the Herpes Simplex virus. The heterogeneous etiology of this disease makes a careful virological examination of all cases of acute cerebellar ataxia important.

SUMMARY

One case of acute cerebellar ataxia associated with Herpes Simplex virus infection is reported. The causative connection between this virus and acute cerebellar ataxia has previously not been recognized.

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CASE REPORT

HYPOTHALAMIC HYPERPHAGIA, OBESITY AND DISTURBED BEHAVIOUR IN ACUTE LEUKEMIA

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The incidence of leukemic involvement of the central nervous system has markedly increased since the advent of antileukemic chemotherapy and is now about 20% of all leukemic patients (9), reaching approximately 30% in children (6). Usually the clinical picture consists of headache, vomiting, meningeal signs, papilledema, cranial and peripheral nerve palsies. Rarely peculiar symptoms such as hyperphagia, excessive weight gain and behaviour disturbances are observed. In the case of acute lymphatic leukemia presented herein, these were the only symptoms of severe meningocerebral involvement, confirmed at autopsy

CASE REPORT

A boy four year old, was admitted to the "Kaplan Hospital" in November 1964 because of enlarged cervical lymph nodes, which appeared one month prior to his admission.

On examination, enlarged lymph nodes in cervical, axillar and inguinal regions of both sides were found. The lymph nodes were fairly well defined, firm, not tender The spleen and liver were palpated 3 cm below the costal margin.

Laboratory findings. Hemoglobin 13 9 g/100 ml, leucocytes count 51,000/mm³ with 10% neutrophils, 4% eosinophils, 20% lymphocytes and 66% lymphoblasts. Platelet count 45,000/mm³ Smears of bone marrow were typical of acute lymphatic leukemia Chest X-Ray revealed enlargement of mediastinal lymph nodes.

The course and main hematological data are presented in Fig. 1 Treatment was started with *6-mercaptopurine* (2.5 mg/kg/day) The patient responded well and 3 weeks later the enlarged nodes had almost disappeared and the spleen and liver had receded to the costal margin, no leukemic cells were found in the blood films and the platelets had returned to normal.

At the beginning of January 1965 after remission of one month, the patient was readmitted, showing signs of acute illness and petechiae and ecchymoses on the skin. Once again enlarged tender lymph nodes were found in the cervical, axillar and inguinal regions, together with hepatosplenomegaly Blood count showed hemoglobin 13.6 g/100 ml, platelets 32,000 and leucocytes 46,000 with 45% blasts. Treatment with hydrocortisone (10 mg/kg/day), 6-mercaptopurine (2.5 mg/kg/day) and chloramphenicol (750 mg/day) was instituted and within 2 weeks a second remission had been brought about. Treatment with hydrocortisone, 6-mercaptopurine and chloramphenicol was discontinued, and meticorten (2 mg/kg/day) was introduced.

At the end of January 1965 new changes appeared. Quite suddenly his appetite increased enormously into definite hyperphagia, eating 12 eggs a day and demanding tremendous servings. Excessive weight gain of about 4 kg in two weeks was noted, and he became obese At the same time his behaviour became bizarre, alternating from periods of rage, restlessness and unreasonable crying to periods of frozen immobility Neurological examination and fundoscopy were negative. Treatment with corticosteroids was stopped few days later being thought to be the cause of these new changes.

From the beginning of March, the patient condition deteriorated progressively Edema of hands and legs and hydrothorax were noted, the latter leading to marked respiratory distress. Repeated peripheral blood examination remained however essentially normal In spite of further therapeutic trial with methotrexate (2.5 mg day) the child died 4 ½ months from the date of his first admission.

AUTOPSY FINDINGS

At postmortem the typical picture of acute lymphatic leukemia was found, with generalized involvement of almost all internal organs. On macroscopic examination of the brain no changes were noted, the meninges were thin and translucent, the ventricles not dilated. On microscopic examination however the subarachnoidal space was found filled by lymphocytes and lymphoblasts,

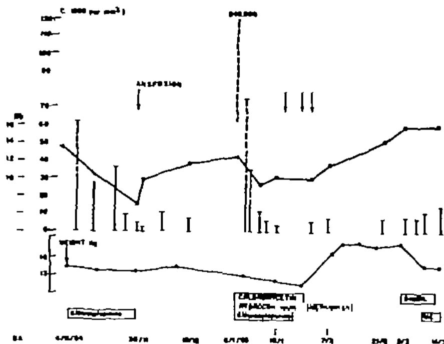


Fig. 1. Hemoglobin levels, leucocyte counts, weight curve and therapy given during the course of the disease. The dashed parts of the vertical lines represent the proportion of immature white blood cells. Diucl = Chlorothiazide; ME = methotrexate.



Fig. 2. Diffuse and perivascular leukemic infiltrations of the medial hypothalamus. (H. and E. 50.)



Fig. 3. Higher magnification of the same region, showing reduced number of nerve cells and their degenerative changes. (H. and E. 176.)

which penetrated into the adjacent brain tissue in the form of perivascular cuffings. These meningeal infiltrations were more extensive at the basal parts of the brain than in the region of the convexities. Diffuse intracerebral leukemic infiltrations were observed in the floor and lateral walls of the third ventricle involving the nuclei of the medial hypothalamus. In these hypothalamic nuclei many nerve cells disappeared and those that remained revealed degenerative changes (Figs. 2 and 3). Diffuse leukemic infiltrations of various intensity were also seen in the region of the optic chiasma, the mammillary body the pons and among the fibers of the trigeminal, facial and acoustic nerves.

DISCUSSION

The presence of unusual hyperphagia, marked obesity and behaviour disturbances in the present case was first thought to be related to the treatment with corticosteroids. The autopsy finding, however of severe leukemic infiltration of the hypothalamus indicates a more pl[illegible] mechanism.

As is well known (2) the hypothalamus possesses the mechanism controlling feeding behaviour. Its more lateral area is designated as "feeding center" the more medial a satiety center". These centers have probably the function of facilitating and inhibiting of feeding reflexes. Experimentally produced lesions in the lateral hypothalamus resulted in aphagia, and lesions in the medial hypothalamus, especially in or near the ventromedial nucleus, resulted in increased food intake and obesity. This hyperphagia was assumed to be a release-phenomenon brought about through destruction of an inhibitory mechanism.

The development of this "hypothalamic syndrome" in cases of acute lymphatic leukemia is apparently rare. Only 14 cases presenting this

Table 1. *Hypothalamic syndrome" in cases of acute leukemia*

Authors	Age (years)	Sex	Type of leukemia	Stage	Treatment with corticosteroids	Clinical features	Leukemic involvement of C.N.S. (at autopsy)
Sansone (1954) [8]	9	M	Ac. lymphatic	Remission	N	Obesity meningeal symptoms and signs	Meninges, hypophysis, olfact. bulb, optic chiasma, tracts and nerves
Sansone (1954) [8]	12	M	Ac. lymphatic	Remission	4 months before complication	Obesity (25 kg), meningeal symptoms and signs	Not performed
De-Toni (1954) [4]	2	M	Ac. lymphatic	Not stated	N	Obesity (25 kg), hypogenitalism	Hypothalamus, hypophysis
Hattory et al., cited by Fortuna (1954) [5]	5	F	Ac. lymphatic	Remission	Not stated	Obesity hirsutism	Hypothalamus
Sullivan (1957) [10]	4	F	Ac. lymphatic	Remission	No	Obesity headache, vomiting	Hypophysis, tentorium, anterior lobe of hypophysis
	4	M	Ac. lymphatic	Remission	No	Obesity drowsiness, strabismus	Not performed
Albin (1958) [1]	6	M	Ac. myeloid	Not stated	Not stated	Obesity (12 kg)	Meninges, hypophyseal stalk, hypothalamus
Zuelzer et al (1960) [11]	7	F	Ac. lymphatic	Remission	Prednisone 3 weeks initially	Hyperphagia, obesity	Not performed
Hughes et al. (1965) [6]	+ 4 cases		Ac. lymphatic	Remission	No	Papilledema, hyperphagia, obesity	Not stated
Shaw et al. (1960) [9]	5	M	Ac. lymphatic	Not stated	Not stated	Hyperphagia, obesity behaviour disturbances	Hypothalamus
Bastrup-Madsen et al. (1963) [3]	3	M	Ac. lymphatic	Remission	4 months before	Obesity disturbed behaviour	Medulla oblongata, mesencephalon, hypothalamus

complication have been reported up to date (Table 1)

On analysis of these cases certain clinical and pathological features emerge. The "hypothalamic syndrome" nearly always occurred in cases of acute lymphatic leukemia, prevalently among boys from 2 to 12 years of age. The weight gain, when stated reached from 4 to 25 kgs during the disease. In the majority of the cases it became apparent during hematological remission. This may be due to the blood-brain barrier which does not allow the antileukemic drugs to reach effective therapeutic levels in the cerebrospinal fluid (3 6) In most of the cases corticosteroid therapy was not applied thus giving strong support to the assumption that these agents do not contribute to the appearance of the "hypothalamic syndrome" In most cases other neurological and endocrinological changes were noted, while in few it appeared as a single sign of meningo-cerebral leukemic involvement. At autopsy examination the whole or part of the hypothalamus was found infiltrated by leukemic cells and very often the meninges and other parts of the brain were involved as well. The lack of additional neurological signs observed in some cases may be explained by the uneven distribution of the infiltrations, which in such cases were more extensive in the hypothalamus than in the other parts of the brain. It must be stressed however that according to the recent experiments by Mabel *et al* (7) increased ventricular pressure alone causes hyperphagia in normal rats, due to its effect on the intact ventromedial area of the hypothalamus.

It may be then concluded, that this hypothalamic syndrome might be the single sign of cerebral involvement in children suffering from acute leukemia. Its recognition can be of value in the early diagnosis and correct treatment of such complications, and may also avoid unnecessary interruption of treatment with corticosteroids.

SUMMARY

Hyperphagia with excessive weight gain and behaviour disturbances without other neurological signs appeared during a remission of acute lymphatic leukemia in a 4 year old boy At autopsy in addition to leukemic involvement of almost all organs, extensive infiltrations were noted in the medial hypothalamus, the meninges and in some other parts of the brain. 14 similar cases collected from the literature were reviewed. The conclusion was drawn, that the "hypothalamic syndrome" may be the only sign of extensive cerebral leukemic involvement. Its recognition may be of value in early diagnosis and correct treatment of cerebral leukemic complications.

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CASE REPORT

CONGENITAL ADRENAL HYPERPLASIA WITH 11 HYDROXYLASE DEFICIENCY

A Case Report and Contribution to Diagnosis

W Blunck and J R. Bierich

From the Department of Pediatrics, University Hospital, Hamburg-Eppendorf Germany

In 1955 Eberlein & Bongiovanni postulated deficiency of steroid-11-hydroxylase as a possible cause of the form of congenital adrenal hyperplasia (CAH) with hypertension, which had been first described by Wilkins and co-workers in 1951 A compilation of the symptoms of 36 reported cases of CAH with 11-hydroxylase deficiency has been made by Gabrilove *et al.* (7). It is clear that hypertension is not an obligatory symptom of the disease, and that in some cases the symptoms only develop when the patients reach adulthood. As opposed to the congenital adrenal hyperplasia with 1-hydroxylase deficiency in the case of 11-hydroxylase deficiency the excretion of reducing steroids is elevated, since 21-hydroxylation is unimpaired and the steroids 11-deoxycortisol and 11-deoxycorticosterone, accumulated due to the enzyme deficiency have an α-ketolic side-chain. The excretion of Porter-Silber-chromogens is raised as well, for the substances tetrahydro-11 deoxycortisol (THS) and the unreduced 11-deoxycortisol (substance "S") are excreted in increased quantities in the urine. On the other hand, the excretion of pregnanetriol is usually only slightly elevated. Due to the variable symptoms in the 11 hydroxylase deficiency the biochemical findings of which are not typical for the classic CAH with 21-hydroxylase deficiency it is difficult to differentiate this condition from an adrenal tumour without detailed diagnostic techniques. The diagnosis can be confirmed by demonstrating a reduction of the excreted THS under Dexamethasone.

CASE HISTORY

The parents of the Turkish boy Volkan E are first cousins. The boy is the parents' first child. The birth was three weeks earlier than the calculated date; the birth-weight was 2900 g. At first the child' progress as normal; there ere no signs of the salt-losing-syndrome. Later on, however remarkably rapid growth-velocity and virilization became evident The excretion of 17 ketosteroids was raised to –4 mg day

A first clinical investigation showed no reduction of 17-ketosteroids under treatment ith Dexamethasone. Because of suspected adrenal carcinoma the child was to be transferred to the surgical department of the University Hospital, Hamburg-Eppendorf, but was instead admitted t our clinic for further investigation.

At the time of admission the boy was years and months old. Body height was 103 cm (11 9 cm abov the mean height of Hamburg boy of this age) Body eight was 17.5 kg. The most remarkable physical symptoms were: normal muscular development, relatively large penis, small infantile testes, hypertrichosis and growing pubic hair (Fig. 1). The blood pressure was between 110/70 and 100/80 mm Hg The skeletal development of the hand, according to Greulich & Pyle, approximated to that of an 8-year-old boy The concentration of sodium in the serum, at 150 mEq/l, was at the upper normal limit; potassium in the serum was, at 3 8 mEq/l, rather low In general the boy was psychologically difficult, restless and sullen.

The excretion of 17-ketosteroids in the 4-hour urine was elevated on the average to 2.7 mg (normal range for age: 0 6–1 0 mg/day) and the average excretion of Porter Silber-chromogens was –1 mg/day (normal range for age: 0 8–1.5 mg/day). A Dexamethasone suppression test (11) was carried out. After treatment with 0 84 mg Dexamethasone for tw days the excretion of the corticosteroids had not decreased. The 17-ketosteroids showed decrease to 1 2 mg day After the twice-daily administration of four-fold doses the 17-ketosteroids and the Porter Silber-chromogens fell to 0.75 mg day At 0 4 mg/day pregnanetriol was at the upper limit of the normal range, but not clearly pathological. The excretion of the different steroid-metabolites is shown in Table 1.

Fig. 2 shows the position of the enzyme-defect, and the excretion of the different steroid-metabolites in healthy children. The nature of the deficiency can be determined by comparing the different patterns. There is high

complication have been reported up to date (Table 1).

On analysis of these cases certain clinical and pathological features emerge. The "hypothalamic syndrome" nearly always occurred in cases of acute lymphatic leukemia, prevalently among boys from 2 to 12 years of age. The weight gain, when stated, reached from 4 to 25 kgs during the disease. In the majority of the cases it became *apparent during hematological remission. This* may be due to the blood-brain barrier which does not allow the antileukemic drugs to reach effective therapeutic levels in the cerebrospinal fluid (3 6). In most of the cases corticosteroid therapy was not applied thus giving strong support to the assumption that these agents do not contribute to the appearance of the hypothalamic syndrome In most cases other neurological and endocrinological changes were noted, while in few it appeared as a single sign of meningo-cerebral leukemic involvement. At autopsy examination the whole or part of the hypothalamus was found infiltrated by leukemic cells and very often the meninges and other parts of the brain were involved as well. The lack of additional neurological signs observed in some cases may be explained by the uneven distribution of the infiltrations, which in such cases were more extensive in the hypothalamus than in the other parts of the brain. It must be stressed however that according to the recent experiments by Mabel *et al* (7), increased ventricular pressure alone causes hyperphagia in normal rats, due to its effect on the intact ventromedial area of the *hypothalamus.*

It may be then concluded, that this hypothalamic syndrome might be the single sign of cerebral involvement in children suffering from acute leukemia. Its recognition can be of value in the early diagnosis and correct treatment of such complications, and may also avoid unnecessary interruption of treatment with corticosteroids.

SUMMARY

Hyperphagia with excessive weight gain and behaviour disturbances without other neurological signs appeared during a remission of acute lymphatic leukemia in a 4 year old boy At autopsy in addition to leukemic involvement of almost all organs, extensive infiltrations were noted in the medial hypothalamus, the meninges and in some other parts of the brain 14 similar cases collected from the literature were reviewed. The conclusion was drawn, that the "hypothalamic syndrome may be the only sign of extensive cerebral leukemic involvement. Its recognition may be of value in early diagnosis and correct treatment of cerebral leukemic complications.

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birth. There were no complications during gestation or at parturition. At three weeks of age, he developed severe vomiting and at one month of age was admitted to the hospital at Langley Air Force Base in Virginia. On admission serum sodium was 118 mEq/l, potassium was 8 0 mEq/l (hemolyzed sample) and CO_2 was 21 mEq/l. A 24-hour urine specimen revealed 4 4 mg of 17 ketosteroid and 1 6 mg of pregnanetriol. The diagnosis of congenital adrenal hyperplasia was made and the patient discharged on 12.5 mg of cortisol per day monthly injections of .5 mg of desoxycorticosterone (DOC) trimethylacetate, and added salt to the diet (approximately 2–3 g per day).

The patient did well following initial diagnosis with normal growth and development through the first year of life. Cortisol dose was increased to 15 mg per day at 1 year of age. At age 1 $^{2}/_{12}$ a systolic blood pressure of 170 mm precipitated his first Clinical Center NIH admission. Hypertension slowly remitted after elimination of DOC. Following baseline studies 3-day 5-day and 17 day courses of adrenocorticotropin[1] (ACTH) were given to assess adrenal responsiveness. Each of these courses resulted in negligible changes in urinary 17-hydroxy and 17-ketosteroids with pregnanetriol levels of 0.3–0 4 mg per 24 hours (values which were close to the blank of the method) In addition, electrolyte changes suggested sodium retaining steroid deficiency The patient was discharged on dexamethasone (Decadron®) 0.75 mg per day and fludrocortisone (Florinef®) 0.05 mg per day Diagnosis was stated only as adrenal cortical hypofunction as evidence for CAH could not be confirmed.

At age 2 /$_{12}$ ACTH was administered at home by the mother for a period of 21 consecutive days. Urine collected at the end of that period showed no measurable androsterone, etiocholanolone, or dehydroepiandrosterone. There was, again, no evidence of CAH.

A third attempt at ACTH stimulation was made at age 3 $^{1}/_{12}$. Corticotropin[2] was given by the local physician for 22 days followed by 3 days of such treatment in the hospital. Seventeen-ketosteroid determinations done on the last 3 days of ACTH treatment ranged from 1 7–.5 mg per 24 hours. Though these results were the highest steroid values obtained between the ages of 1 and 4 they represented a clearly subnormal response to tropic stimulation. Seventeen-hydroxy steroids were not measured after the third ACTH course due to laboratory error

The patient continued to do well while on replacement medication for hypoadrenalism though growth rate decreased after 1 /$_{2}$ years of age. Because of retarded bone age and a height less than the third percentile, cortisol was slowly reduced from 15 mg per day at age 2 /$_{12}$ to 6 mg per day at age 5 /$_{12}$. At age 6 a striking growth spurt was noted with a concomitant maturation of bone age (see Table 3). Mother also reported some increase in penile size though no pubic or axillary hair had appeared.

At age 6 $^{11}/_{12}$ the patient was admitted to the Clinical Center again for diagnostic confirmation of what clinically appeared to be classical congenital adrenal hyperplasia. The laboratory data bore out this suspicion as total 17-ketosteroids, pregnanetriol and pregnanetriolone were elevated while off replacement (Table 1). Dexamethasone suppression and ACTH[2] stimulation resulted in responses consistent with CAH (Table 2). Bone age had reached the 10 year level.

The patient is presently nearing his eighth birthday and has been adequately maintained on 15 mg of cortisol per day Since salt-losing tendency was mild, sodium retaining steroid was discontinued. Significant laboratory results and growth data are given in Tables 1 ., and 3

COMMENTS

Documented congenital adrenal hyperplasia followed by adrenal unresponsiveness with subse

Table 1 *Urinary steroids determined in patient at age 6 /$_{12}$ kile ff all treatment*

Glucuronides (mg day)		Sulfates (mg/day)	
Androsterone	1 4	Dehydroepiandrosterone	0 23
Etiocholanolone	0 7	Δ^5-pregnenediol	0.64
17-OH-pregnanolone	5 1	Δ^5-pregnenetriol	0.51
Pregnanetriol	6 0		
Pregnanetriolone	14 8		

[1] As H. P. Acthar gel, Armour Pharmaceutical Company Kankakee, Illinois.

[2] Cortrophin-Zinc, Organon, Inc West Orange, New Jersey

Table 2. *Adrenal function studies performed on patient from age 1 2/12*

Age	Adrenocorticotropin stimulation	Glucocorticoid dose (mg/24 hrs)		Urinary 17-OH steroids* (mg/24 hrs)	Urinary 17-ketosteroids (mg/24 hrs)	Pregnanetriol (mg/24 hrs) or other urinary steroids measured
1–2/12	None	Cortisol	15	1 1	0.5	
				1.5	0.5	
				1 6	0.6	
				1 2	0.6	
1 3/1	Baseline	Decadron	0.75	0.2	0.5	
	10 units I. M. b. i. d. × 3 days	Decadron	0.75	0 1	0.5	
	10 units I. M. b. i. d. × 3 days	Decadron	0.75	0.5	2.9	
	After 1 week off ACTH	Decadron	0.75		0 4	
	10 units I. M. b. i. d. × 4 days	Decadron	0.75	0.2	0.6	
	4 more days of 10 units I. M. b. i. d.	Decadron	0.75	0.1	0.8	
	6 more days of 10 units I. M. q. d.	Decadron	0.75	0.1	0.3	0.3
	2 more days of 10 units I. M. q. d. and 1 day of 25 units I. V. over 8 hrs for 17 days total of ACTH	Decadron	0.75	0.5	0.6	0.4
	Day after above	Decadron	0.75	0.1	0.5	0.3
1–8/1	None	Cortisol	15	2.4	0.8	
2–2/12	20 units I. M. q. d. 21 days, urine collected on three following days	Cortisol	15			N androsterone, etiocholanolone, or dehydroepiandrosterone
3–11/12	20 units I. M. q. d. 22 days	Cortisol	8			
	20 units I. M. on 23rd day	Decadron	0.75		1 7	
	20 units I. M. on 24th day	Decadron	0.75		1 6	
	40 units I. V. on 25th day	Decadron	0.75		2.5	
6–11/12	None	None		75.2	19 6	26 (See Table 1 for other steroids measured)
	None	Decadron per day 4 days	1 0 mg	6.2	18.2	
	20 units I. M. given day after above Decadron	Decadron	0 75	10.8	55.2	
	None	Decadron per day 7 days	2.0 mg	3.5	6.6	

* Measured by method of Silber & Porter [8] until age 6–11/12 after which time the Few procedure [10] was used.

quent reappearance of the signs of enzyme deficiency is a bizarre history indeed. Attempts at explaining these events are not at all satisfactory. The most attractive unitary hypothesis is that long-lasting, but reversible suppression of ACTH (with secondary adrenal atrophy) by exogenous corticoids produced the Addisonian picture, a sequence which has not previously been described in congenital adrenal hyperplasia.

There is little question that hypoadrenalism was present in this patient between the ages of 1 and 4. Following prolonged courses of ACTH administration at three different and widely separated periods in his life subnormal adrenal responses were recorded. At age 3 11/12, 24 days of intramuscular ACTH followed by an intravenous dose on the 25th day resulted in only minimal increases in 17-ketosteroids. These data are even more striking when comparisons are made with the expected 17-ketosteroid values (4–30 mg per 24 hours) for the untreated adrenogenital syndrome at this age (9).

Steroid replacement therapy was started at age 5 weeks with a dose not unusual for this age group (cortisol, 12.5 mg per day), and increased to 5 mg of cortisol 3 times daily at the end of the first year of life. Considering that the patient at age 7 1/2 was adequately treated with 15 mg of cortisol per day he may have been receiving a completely suppressive dose during the first 14 months of life. With the exception of hypertension there was, however no clinical evidence of steroid excess.

Since hypertension slowly diminished following

Table 3. *Growth data and steroid replacement dosages from the time of patient's first NIH admission*

Chronologic age	Height (percentile)[a]	Bone age[b]	Glucocorticoid dose (mg/24 hr)		Florinef dose (mg/24 hrs)
1 2/12	10		Cortisol	15	None (in hospital)
1 3/12	3–10	1 3/12	Cortisol	15	None (in hospital)
1–4/12	10		Cortisol	15	0.05
1 5/12	3		Decadron	0.75	0.05
1–8/12	< 3	1 3/12	Cortisol	15	0.025–0.05
1 11/12	< 3	1–3/12–			
		1–6/12	Cortisol	15	0.05
2 3/12	< 3		Cortisol	12	0.05
–4/12	< 3		Cortisol	10	0.05
2 7/12	< 3	2–6/12	Cortisol	9	0.05
3–8/12	< 3	–8/12	Cortisol	8	0.05
4–3/12	< 3		Cortisol	9	0.05
5–3/12	< 3	2–8/12	Cortisol	9	0.05
5–4/12	< 3		Cortisol	6	0.05
5–9/12	< 3	3	Cortisol	6	0.05
6–3/12	10	6–3/12	Cortisol	6	0.05
6–10/12	3		Cortisol	6	0.05
6–11/12	50	10	Cortisol	8	0.05
7 5/12	50	10–1/2	Cortisol	15	0

[a] Anthropometric chart of the Childrens Hospital Medical Center, Boston, Massachusetts.
[b] By the standards of Greulich & Pyle.

withdrawal of the sodium retaining steroid, DOC was felt to be the cause of the increased blood pressure. However the dose of DOC (and supplemental salt) was modest, and, even given to a normal individual of this age might not be expected to cause the degree of hypertension produced. It is possible, then, that this patient may have been particularly sensitive to both mineralo- and glucocorticoids. It is striking that increased endogenous ACTH effects (with resultant signs of CAH) appeared only after cortisol was reduced from 15 mg per day to 6 mg per day over four year period during which body size increased (see Table 3). Bone age matured dramatically following this reduction in steroid dose with a gain of 3 years in only 5 months time.

Growth retardation following this patient's first hospitalization may also be related to exogenous steroid administration. Doses of glucocorticoid sufficient to completely suppress adrenal function have been shown to significantly reduce growth hormone levels (7).

Other explanations for the unusual series of events described may also be proposed. A postnatal adrenal injury due to either trauma or infection could have produced Addison disease in the patient, but there is no indication that such an event occurred. ACTH resistance has been described in a small number of patients by Migeon et al. (5) but transient resistance has not been documented.

SUMMARY

A 7 1/2-year-old boy in whom the diagnosis of congenital adrenal hyperplasia was made at age 5 weeks is presented. Treatment with exogenous corticoids was started immediately but between ages 1 to 4 an Addisonian state intervened. Classical congenital adrenal hyperplasia then reappeared after age 6 following a reduction in corticoid replacement. Suppression of ACTH by exogenous glucocorticoid is suggested as a possible cause of this reversible adrenal failure.

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PROCEEDINGS OF PEDIATRIC SOCIETIES

DANISH PEDIATRIC SOCIETY

Meeting, March 10, 1967

Henning Andersen: *Hormone therapy of undersized and too tall children*

An account is given of the effects of various hormones on both the growth in length and on the development of the centres of ossification. Various conditions involving the growth hormone are mentioned, its extraction and clinical employment, illustrated by the author's clinical experience.

Finally an account is given of oestrogen treatment of girls with unusual growth in length. The author's experience and that of other authors appear to show that provided these girls (who do not show any endocrine abnormalities) are treated before the prepubertal growth spurt commences, i.e. at the age of 9–10 years, the final height can be reduced. Unfortunately parents often seek medical advice for these children too late, i.e. after the greater part of the growth in length has occurred and after menstruation has commenced. In cases such as these, very little or nothing at all can be achieved. The final result is difficult to assess as there is no reliable method of predicting the final height in children, particularly when they deviate from the average heights. The treatment is carried out as cyclical injection therapy with depot preparations of estrogens and progesterone. According to the experience hitherto obtained, it has not resulted in any immediate discomfort nor disturbances of menstruation after cessation of treatment.

Discussion

E. Thamdrup mentioned the effect of medroxyprogesterone in the treatment of premature puberty. The preparation is capable of inhibiting feminisation of girls and they do not become virilised. No changes are observed in boys. The preparation cannot inhibit the increased growth in length and devopment of bones. It is important to commence treatment of too tall girls at the correct time.

P. Plum emphasized that it is probably just as mentally traumatising for children to be too tall as it is to be too small. He had previously treated a few tall girls with estrogens. One of them was treated from about the age of 12 years and with apparently good result.

Ingrid Thorn & Else Andersen: *Echo-encephalography*

Echo-encephalography has been employed in the Children's Hospital, Fuglebakken, since June 1965 and over 100 investigations have hitherto been undertaken. In 15 hydrocephalic patients, results of simultaneous pneumo-encephalography were available and good agreement was found between Evans's ratio measured in the pneumo-encephalogram and the index measured in the echo-encephalogram.

It is concluded that echo-encephalography is a valuable screening test in the differential diagnosis between hydrocephalus and macrocephaly in children under the age of one year. The method is sufficiently sensitive to register alterations in the size of the ventricles and it may therefore be of value in establishing the indications for operation in hydrocephalic patients. In certain cases, the investigation may reveal space-occupying intracranial processes. The method is relatively rapid, reproducible, painless and non-dangerous. It cannot replace pneumo-encephalography but can probably reduce the number of such examinations.

Discussion

J C Melchior Can dilatation of the lateral ventricle in infants be demonstrated by the method and can subdural hematoma be demonstrated?

Ingrid Thorn. Dilatation can be demonstrated as a rule. There were no cases of subdural hematoma in our material, but Iréne Sjögren states that such cases are difficult to demonstrate with the method.

P Plum. How long does the investigation take?

Ingrid Thorn. As a rule, about half an hour

Sv Brandt Can the method be employed to follow the effects of operation for hydrocephalus?

Ingrid Thorn. Yes, but only until about the age of two years.

E. Terslev *A case of an abnormal muscular bundle in the right ventricle*

In a boy aged $5\,^1/_2$ months with a moderate systolic cardiac murmur electrocardiographic signs of right-sided axisdeviation and right-sided ventricular hypertrophy cardiac insufficiency developed and led to sudden death.

At autopsy no evidence of septal defects, stenosis of the ostiae, transpositions or anomalies of coronary arteries was found. There were no signs of essential pulmonary hypertension. On the other hand, a very pronounced thickening of the walls of the right ventricle was observed, and an abnormal muscle bundle was found in the conus pulmonalis.

Discussion

Sv Heinild inquired whether there had been any noxious agents in the second month of pregnancy to which *E. Terslev* replied that no relevant information was available.

Birgit Pettersen *Quantitative determination of the total protein and gamma globulin in breast milk*

The material comprises 150 samples of milk, collected during the first eight months of lactation and seven samples of colostrum from the first 48 hours of lactation and, finally isolated samples from $13\,^1/_2$ 15 months after partus.

The total protein was determined according to Lawry's method. In colostrum, the average value was found to be 3 7 g/100 ml. The protein content fell rapidly and from one month after partus and onwards, it remained about 1 1 1 4 g/100 ml.

The gamma globulin content was determined by the immune precipitation technique. The average value in colostrum was found to be 20.2 mg/100 ml and this decreased only gradually during the subsequent months of lactation. There were great individual variations in the material. The values were independent of the quantity of milk produced.

Knud E. Petersen. *Nephrogenic diabetes insipidus*

A male infant had fever for several months while his general condition remained uninfluenced and no focus of infection could be demonstrated. The diuresis and specific gravity of the urine were finally measured after a high serum sodium had been observed. A diagnosis of diabetes insipidus was verified by means of a thirst test in which no antidiuresis occurred. The patient was treated with fluid *ad libitum*, a low-sodium diet and a thiazide preparation.

In the parents and a sister aged four years, the Carter Simpkiss thirst test revealed that only the father concentrated the urine normally This observation is in agreement with the assumption that the mode of inheritance of the disease is sex linked, incomplete, dominant with variable penetration in the female carriers.

Several members of the father's family had hemolytic anemia. It is known that patients with sickle-cell anemia frequently suffer from vasopressin-resistent diabetes insipidus. It did not prove possible to demonstrate any connection between the predisposition to hemolysis in this patient and his nephrogenic diabetes insipidus

Flemming Holck. *E coli 26 enteritis in children treated with nalidixic acid*

Eighteen children with enteritis caused by E coli 26 were treated with nalidixic acid (Negram[1]) with a dosage of 60 mg/kg/24 hours for seven days and were compared with a corresponding number of patients with the same infection treated with nitrofurantoin (Furadantin[2]) The great majority of the patients were under the age of one year but the patients treated with nalidixic acid

were more severely ill than those treated with nitrofurantoin.

Approximately 3/4 of the patients in both of the materials were bacteriologically cured and all of them were clinically cured and no side-effects were observed. In contrast to Furadantin, it proved easy to get the children to take Negram orally in the form of the Pediatric Suspension.

Meeting, April 19 1967

P Fogh-Andersen. *Pierre Robin' (Lenstrup) syndrome*

The triad of micrognathia, glossoptosis and, as a rule, cleft-palate is a syndrome which has long been recognised as the cause of respiratory and feeding difficulties in infants. It was first described by Lannelongue & Menard (1891) among others. A series of publications by Pierre Robin including a monograph in 1929 resulted in his name being connected with the syndrome although he did not emphasize cleft-palate as a component. In Scandinavia, particularly in Denmark, the syndrome could rightly be termed Lenstrup's syndrome after the Danish pediatrician Ejnar Lenstrup who described it in 1926, apparently independently of Robin. Lenstrup published a report of three cases from Queen Louise's Children's Hospital. All three patients had cleft palate and Lenstrup recommended to place the patient on the side or in the prone position as an effective treatment.

It is a common observation that there is a tendency to spontaneous improvement once the first critical period has elapsed. In severe cases, particularly where the tongue shows a tendency to become incarcerated in the accompanying cleft plate, early operative treatment may be necessary in order to hold the tongue forward.

As a result of the centralised treatment of children with hare-lip and cleft-palate in Denmark, we have collected a number of infants with Robin-Lenstrup's syndrome admitted to The Deaconess Hospital in Copenhagen in recent years. In the past decade, there were 18 cases and of these nine patients required operative treatment in infancy with tongue-lip fixation. One of these patients died from pneumonia while the rest recovered and could be discharged for readmission some months later for division of t fixation. The mandible developed surprisingly ell and a Wardill plastic operation for closure the cleft palate could be carried out at the age of two years.

Discussion

B. Friis-Hansen. What about the feeding of the infants after operation?

P Fogh-Andersen. There are no particular difficulties and the mandible grows forwards, although we do not quite know why

Grethe Holst From a dentist's point of view I wonder whether it is right that the operative treatment is not completed in these children until the age of two years.

P W Brestrup Were chromosome investigations undertaken in these patients?

P Fogh-Andersen. No abnormalities were demonstrated in the patients investigated.

Henning Andersen By means of special mechanical devices, prone position and Elastoplast cap suspension of the head, operation can probably be avoided in a number of cases.

P Fogh-Andersen. *Feeding of infants with cl ft palat*

In Denmark, all newborn infants with hare-lip and/or cleftpalate are registered at the N tional Institute for Speech Therapy (Copenhagen or Ar hus), and are summoned for operation to the Deaconess Hospital in Copenhagen at the age of two months for treatment of the hare-lip and t the age of two years for treatment of cleft-palate.

About one fourth of these children ha e hare-lip only and will be able to breast feed normally Nearly three fourths, however have cleft-palate with or without accompanying hare-lip and will only be able to take the breast in exceptional cases As a rule, they must be fed by spoon, tube or bottle or by other means preferably with breast milk

Many devices have been recommended to aid feeding, particularly teats of special shapes and forms A spoon and an ordinary baby's bottle may also be of use when the spoon is held on the infant's tongue and milk is poured into the spoon in small quantities from the bottle. A cheap and ef

fective combination of spoon and bottle is manufactured by the Danish plastic factory Rosti. By means of pressure on the bottle, suitable quantities of milk can be introduced via the bowl of the spoon into the infant's mouth. For a number of years, this has been the standard method in the Deaconess Hospital and it can be recommended as the standard procedure, particularly for home use.

In special cases, a little prosthesis can be made for temporary closure of the cleft-palate until the time for operation. In cases of more serious feeding difficulties or other complications, particularly if other serious malformations are present, it is advisable to have the infant admitted to a pediatric department.

Discussion

P W Brestrup During the past 15 years, we have employed an angled spoon for feeding infants with hare-lip and cleft palate.

B Friis-Hansen. Disposable bottles must be regarded with a certain scepsis, partly for hygienic reasons and partly because plastic which can tolerate heat has a tendency to produce toxic products.

Cooke, M Lunding, N F Lomholt, M. Yssing, B Zachau-Christiansen & B Friis-Hansen. *Respirator treatment of newborn infants*

Published in *Acta Paediat Scand 56* 498, 1967

E. Hippe & Torben Iversen. *Familial ectodermal dysplasia with increased serum thyroxine*

Published in *Acta Paediat Scand 56* 687 1967

Discussion

K. Siersbæk-Nielsen. I have had the opportunity to examine the protein-bound serum thyroxine in two families with anhidrotic ectodermal dysplasia and have found the serum thyroxine to be normal. In the case reported by Hippe & Iversen, there is probably a random combination of anhidrotic ectodermal dysplasia and familially raised thyroxine-binding globulin in the blood.

Meeting, June 10, 1967

The new pediatric department in the Frederiksborg County Central Hospital in Hillerød was demonstrated.

Birthe Lund & Margareta Mikkelsen. *Partial trisomy syndrome*

A male infant was admitted to the department at the age of ten days on account of cleft-palate. There was a family history of hare-lip, reduction of hearing and encephalopathy At the age of 15 months, the boy was severely retarded both motorically and mentally The ears were large and low placed, the chin receding and a cleft-palate was present. Dental eruption was delayed but rudimentary teeth were present. Further there was thoraco-lumbar scoliosis, poor muscular development in the shoulder regions and hyperextensible joints in hands and fingers but no transverse palmar creases. Muscular tone was found to be increased in the lower limbs. The scrotum was underdeveloped and the penis short and adherent to the scrotum. An atrial septal defect with left right shunt was demonstrated. Urography and cystography during micturition showed a pouch which probably was an utriculus prostaticus, measuring 3 × 6 mm. Radiography of the skeleton, cranium and centres of ossification revealed normal conditions. An EEG was grossly abnormal with focal activity at the age of eight months but normal at 15 months. Pneumoencephalography revealed central atrophy Fetal hemoglobin could not be identified. The quantity of haemoglobin A_2 was normal and no abnormal fractions could be demonstrated. Morphology of the leucocytes was normal. Chromosome investigations revealed 47 chromosomes. The extra chromosome belonged morphologically to the 21-22 Y group. Autoradiographic studies showed that the extra chromosome concluded DNA synthesis earlier than the majority of the other chromosomes. An extra Y chromosome and a deleted X chromosome could thus be excluded. As the extra chromosome was apparently not satellite-carrying or in satellite-association with other acrocentric chromosomes, the most probable cytogenic diagnosis is a deleted chromosome no. 17 Deletion has affected the long arm while the short arm and the centromere region have been retained. Sex-chromatin determinations did

not reveal any Barr bodies. Chromosome investigations on both parents showed normal conditions. Clinically the patient shows some symptoms compatible with the 13–15 trisomy syndrome and others which fits into the 17 18 trisomy syndrome. The clinical findings are also in agreement with partial 13–15 and 17 18 trisomy syndromes. The genital malformation is charac teristic for the 13–15 trisomy but the malforma tions of the eyes, polydactyly hæmangiomata, the abnormal leucocyte morphology and the raised content of fetal hemoglobin characteristic of the syndrome are absent. It is not possible to determine whether the cleft-palate is determined by the extra chromosome material or is genetically conditioned in another manner

K. Hauge Kristensen. *Pelizaeus-Merzbacher's syndrome*

A boy aged 2 1/ years with Pelizaeus-Merzbacher' syndrome was described. A brother aged 11 years had also had the same symptoms. There were a healthy brother and three healthy sisters in the family Pneumoencephalography revealed left-sided central atrophy Biopsy from a peripheral nerve showed myelin degeneration and biopsy from the brain showed sudanophile gra nules along the neurites. Increased content of glycolipid fractions were demonstrated in the urine, serum and cerebro-spinal fluid. The urine showed lowered arylsulphatase a and b values.

Discussion

E. Thamdrup· Does peripheral nerve biopsy not give just as much information as brain biopsy in these cases?

J Melchior· No, not in the disease mentioned here but it is sufficient in metachromatic leucodystrophy

Hanne Sand. *Goodpasture syndrome*

A girl aged 1 years was admitted on account of cough and subfebrile condition which had been present for about six months. In addition to hem optysis and hematuria, hemorrhages int the skin and attacks of abdominal pain were noted.

No evidence of collagenosis was encountered as judged by serum reactions and skin and muscl biopsy Renal biopsy showed glomerulonephritis. ASH was raised and cold agglutinins were found. Cultures for bacteria and viruses were negative and there was no eosinophilia. The patient was treated with large doses of steroids with doubtful effect and with antibiotics.

The patient was transferred to the Dialysis De partment in the Rigshospital on account of uremia, but death occurred from pulmonary insufficiency Autopsy revealed Goodpasture s syndrome and panarteritis. On account of the re semblance to Schoenlein s purpura, virus allergy was considered as an etiological possibility

The disease may be compared with idiopathic pulmonary hemosiderosis (IPH). The clinical pic tures cannot be differentiated. There is possibly an age-determined transition from IPH to Good pasture s syndrome to periarteritis nodosa. Treat ment with steroids and cytotoxic drugs may be of value.

J Ramsøe Jacobsen. *Myocarditis in a girl receiving antiepil ptic therapy with phenytoi and phenobarbital*

Ten days after the commencement of antiepileptic treatment with phenytoin, a girl aged five years developed generalized exanthema and pyrexia of 40 C which disappeared a few days after withdrawal of the drug. Treatment with phenobarbital was then instituted and six days later she again developed exanthema with high fever The temperature returned to normal during treatment with antozolin and the exanthema became paler At this point gallop rhythm was demonstrated by auscultation and in the ECG the T waves were found to be flattened and the amplitude of the QRS complex diminished, the heart was found to be slightly enlarged compared with radiographic records from one month previously and the lactate dehydrogenase values were raised. A few days later the T waves were found to be inverted and thereafter the changes gradually became normalized. The patient did not show any signs of decompensation and tachycardia was only present in the initial febrile period. Follow-up investigations during the subsequent years showed normal car diac conditions.

Myocardial involvement as part of a toxic, probably immunological, reaction to phenytoin has only been described on few occasions. In the patient reported here it was not possible to

demonstrate whether the myocardial affection was due to phenytoin or phenobarbital but it was de finitely part of a process which commenced dur ing phenytoin therapy In the literature, it is emphasized that the heart is not uncommonly involved *in immune reactions to drugs, mostly sulphonamides.* Myocardial involvement is frequently subclinical but can be disclosed by repeated auscultation, ECG and radiographic examination of the heart. As the risk of serious arythmia and disturbances of conduction is considerable, it is important to bear in mind the possibility of myocardial involvement in toxic reactions to drugs.

Discussion

J Melchior Was the serum phenytoin determined and was eosinophilia present?

J Ramsøe Jacobsen. These were not investigated.

E Wamberg Was there clinical evidence of myocarditis?

J Ramsøe Jacobsen. No, none apart from the tachycardia.

E. Thamdrup *Russell's diencephalic wasting syndrome*

A female infant aged 13 months was admitted to because of failure to gain weight for the five months. Weight 9 0 kg (approximately 1 kg below the average for the height) length 84 cm. (5–6 cm more than the average for age). The patient was very wasted. The skin on the buttocks and the thighs hung in folds. She looked pale despite a normal hemoglobin. She was extremely active motorically and showed hysterical tantrums. The thyroid activity was normal and no evidence of malabsorption was found. In the course of one month she lost 150 g despite a food intake which was above normal.

On admission to hospital on a second occasion at the age of 18 months a slightly abnormal EEG was found but no abnormalities on clinical neurological investigation and ophthalmoscopy Excretion of 17-ketosteroids and 17-ketogenic steroids in the urine were normal and the pituitary-adrenal function as estimated by the metopirone test was also normal Radiographic examination of the cranium and the centres of ossification showed normal findings. Lumbar pneumoencephalography revealed a suprasellar tumour which filled the anterior part of the third ventricle. At operation, cystic tumour tissue was found under the frontal lobes continuing medially into the brain stem towards the hypothalamus. The greater part of the optic chiasma was replaced by *tumour tissue. The histological diagnosis* was edematous ependymoma with invasion of the optic nerve. The tumour could not be completely re moved. Post-operative irradiation treatment was administered.

Approximately 50 similar cases have been de scribed in the literature. The clinical picture is seen in children between three months and three years. The main manifestation is wasting despite a normal or increased appetite. These children are very active and mentally almost euphoric but neurological symptoms are absent or are very slight. Raised protein levels in the cerebro-spinal fluid are frequently found. Reduced hypophyseal reaction in the metopirone test, hypoglycemia, eosinophilia, serum electrolyte disturbances, increased growth in length, possibly acromegalic features and raised plasma somatotropin are inconstant findings. The latter is perhaps of significance for the extreme loss of fatty tissue.

Discussion

Henning Andersen. The course of the disease is frequently prolonged. Growth is increased and there are signs of lipolysis. An idiopathic form also occurs with signs of lipolysis only

E. Thamdrup The syndrome should be suspected in all infants with anorexia in the first years of life.

Meeting, June 26, 1967

Meeting with the Danish Society for Research into Mental Deficiency

J Lenstrup *An account of the patient material and the principles of examination and treatment of patients in the children's hospital in Vangede*

E. Niebuhr *Clinical demonstrations*

Lise Hauge Berg & E. Wamberg *A socio-medical analysis of newly registered patients for the chil dren's department of the Danish National Service for the Mentally Retarded in Copenhagen*

Torben Iversen

NEW BOOK RECEIVEDS

Askin, John A., Cooke Robert E., and Haller J Alex J (eds): A Symposium on the Child. Johns Hopkins Press, Baltimore, Maryland, 1967 376 pages. Price $ 10.00.

Blauth, W Der longitudinale Femurdefekt. Beilageheft zu Band 103, Z. Orthopädie. Ferdinand Enke Verlag, Stuttgart, 1967 119 pages. Price DM 35

Wolstenholme, G E. W and Porter R. (eds). The human Adrenal Cortex: its Function throughout Life Ciba Foundation Study Group no. 27 J & A. Churchill Ltd., London, 1967 142 pages. Price 22s. 6d.

Ellis, Errington. The Physical Management of Developmental Disorders. Clinics in Developmental Medicine No. 26. W Heinemann Medical Books Ltd., London, 1967. 90 pages. Price $ 2.50.

Rossi, E. and Stoll, E. (eds.): Cystic Fibrosis. Proceedings of the 4th International Conference on Cystic Fibrosis of the Pancreas (Mucoviscidosis), Geigy Symposium, 1966. Part 1. Moderne Probleme der Paediatrie Vol. 10/Bibliotheca Paediatrica No. 86/ S. Karger AG, Basel/New York, 1967. XVI + 404 pages, 102 fig., 80 tab Price sF /DM 95.-

Pedersen, Jørgen. The Pregnant Diabetic and her Newborn. Problems and Management. Munksgaard, Copenhagen, 1967 219 pages. Price DKr 60.

Briand, H E Laplane R., Lafourcade J Mozziconacci, P Rossier A., Royer P and Thieffry S (eds.): Journées Parisiennes de Pédiatrie 1967 Éditions Médicales Flammarion, Paris, 1967 416 pages, 70 fig. Price F 80 00.

Christensen, Erna and Melchior Johannes: Cerebral Palsy—A Clinical and Neuropathological Study Clinics in Developmental Medicine No. 25 W. Heinemann Medical Books Ltd., London, 1967 134 pages. Price 28

Melnick, J. L. (ed) Progress in Medical Virology Vol. 9 S. Karger AG Basel/New York, 1967 XIV + 496 pages, 53 fig., 92 tab. Price sFr./DM 88

Regamey R. H Hennessen, W Ikić, D and Ungar J (eds). International Symposium on Immunological Methods of biological Standardization Royaumont (France). Symposia Series Vol. 4. S. Karger AG, Basel New York, 1967 XII + 380 pages, 130 fig 74 tab Price sF./DM 54.

BOOK REVIEWS

G. E. W Wolstenholme and Ruth Porter (eds.)· *The human adrenal cortex: its function throughout life*

142 pp., illus. J & A. Churchill Ltd., London, 1967 22s 6d.

This booklet is nr 77 in a series published by the Ciba Foundation and contains the proceedings f one-day conference held 1 September 1966 in honour of F Verzar This pioneer in the field f adrenal research gives a historical review of the last 40 years research on the adrenal cortex. The following chapters are devoted to the function of the adrenal cortex during different periods of life starting with the foetus and ending with old age. The adrenocortical response to surgical, and acute medical stress and to chronic disease is dealt with in three different papers. The status of the adrenals in pregnancy is also discussed. For those who want short and concise introduction into the actual problems of adrenocortical function in different situations this booklet is very wellcome. The names of the participants of the conference guarantee a high standard of the papers and stimulating and sometimes also provocative discussion

C G Bergstrand

Ellis, Errington. *The Physical Management f Developmental Disorders, Clinics in Developmental Medicine N 26* pp., illus. W Heinemann Medical Books Ltd., London 1967 $ 2.50.

The author points out how pediatrics in Western Europe and North America has changed towards developmental pediatrics and how the habilitation of handicapped children plays an important part. The pediatrician must here cooperate with other medical specialists, physiotherapists, social workers and psychologists. The author outlines the principles f this work at the Percy Headly Centre where he has treated cerebral palsied children since 1953 Physical treatment, he means is treatment in the strict meaning of the word only when it deals with younger children. Later on it is better characterized as physical care and maintenance and physical education. For the early treatment he prefers the Bobath method The role of the parents in the treatment of these children is pointed out. There also are some examples of the physiotherapeutic treatment in photographs and short description f physical aids.

This book gives an easily read short survey of many of the modern aspects on handicapped children especially those with cerebral palsy It stresses the importance of good teamwork between the many different categories involved in the treatment. It should be read by doctors, physiotherapists and others concerned with cerebral palsied children and perhaps it will bring about a better understanding of each others work.

Ingrid Bjerre

R. H. Regamey W Hennessen, D. Ikić & J Ungar (eds.): *International Symposium on Immunological Methods of Biological Standardization, Royaumont (France)*, Symposia series, vol 4 XII + 380 pp 130 figs., 74 tables. S. Karger AG Basel, New York, 1967 sFr/DM 54, 99s.

This book contains 37 articles on a variety of methods used when assaying biological reactions by immunological methods. The following problems and methods were presented. Immunoelectrophoresis, gel diffusion and chromatographic methods of antibody purification; fluorescent antibodies, isotopes in the study of antigen–antibody reactions; problems in the standardization of allergens and vaccines; standardization of viral antigens and antibodies; haemolytic, haemagglutination and cytotoxic reactions in the studies of cellular antigen.

The articles are frequently designed in order to show possibilities of errors in techniques and are usually followed by an adequate set of references. Discussion of the articles are included. The main virtue f this book is that it brings together very useful technical information about wide collection of immunological methods. It would thus be suitable to have for any routine immunological laboratory serving as handbook and introduction into immunological methods. The main disadvantage as far as active scientists goes is that the book is already bit out of date in certain areas, being from a symposium in Royaumont, France, in 1965

Hans Wigzell

J A. Askin, R. E. Cooke & J A. Haller J (eds.): *A Symposium on the Child*, 376 pp., illus. The Johns Hopkins Press, Baltimore, Md., 1967 $ 10.00

This book contains twenty-three selected essays presented on the occasion f the seventy-fifth anniversary of the Johns Hopkins Hospital and the dedication of the Children Medical and Surgical Center in May 1964 The authors, who are physicians of outstanding reputation, have earlier worked at the Johns Hopkins Hospital The various papers give reviews of wide range of different topics in pediatric medicine and surgery Such subjects as esophageal disorders, transplantation, emergency cardiac surgery in the newborn, surgical management of chest wall deformities and of hypospadias are discussed Aspects of prematurity infant feeding, immunity growth and development, and mental retardation are given. One chapter deals with the history of the Harriet Lane Home, hich is fascinating reading. Besides that the book gives summary f current developments in some topics of pediatric medicine and surgery it is of historical value

Tor Lindberg

Acta Pædiat Scand 57 176 1968

ANNOUNCEMENT

AMERICAN ASSOCIATION FOR CHILD CARE IN HOSPITALS

The annual meeting will be held at the Sheraton Cleveland Hotel, Wednesday May 8, 1968 to Saturday May 11, 1968.

The theme for the conference is "Coping with Stresses of the Child in the Hospital" Morris Green, M. D., Professor and Chairman, Department of Pediatrics, Indiana University will address the meeting May 9th. At dinner meeting on May 10th, Florence Erickson, Ph. D., Professor and Chairman, Department of Pediatric Nursing, University of Pittsburgh, will be the speaker and Albert J. Solnit, M. D., Professor of Pediatrics and Psychiatry Yale University will give the closing lecture on May 11th. Pediatricians, child psychiatrists, psychologists, nurses and child care workers from the United States and Canada will lead panels as well as seminars. A resource center will be open and films shown.

THE TENTH INTERNATIONAL CANCER CONGRESS

The Congress will be held May 22 29 1970, in Houston, Texas under the auspices of the International Union Against Cancer. Secretary General: Murray M. Copeland, M. D., the university of Texas M D. Anderson Hospital and Tumor Institute, 6723 Bertner Avenue, P O. Box 20465 Astrodome Station, Houston, Texas 77025 (cable address Cancongi.

EUROPEAN SOCIETY FOR PAEDIATRIC NEPHROLOGY FOUNDATION MEETING

The foundation meeting of this society took place in Glasgow and was attended by a total of some seventy persons. The society to date, consists of forty-three members from twenty European countries. The founders are Dr Gavin C. Arneil, Department of Child Health, University of Glasgow and Dr Harmen A. W. M. Tiddens, Wilhelmina Children's Hospital, Utrecht, Netherlands.

During the course of the three-day meeting thirty scientific papers were read and the foundation lecture was given by Professor Henry L. Barnett, of the Albert Einstein College of Medicine, New York, U.S.A.

The meetings took place in the Department of Child Health, University of Glasgow at the Royal Hospital for Sick Children, Yorkhill, Glasgow. The receptions to the new society were given by the University of Glasgow and by the Royal College of Physicians and Surgeons of Glasgow. The foundation dinner of the society was held under the auspices of the University of Glasgow and the meeting [illegible] to the [illegible] of [illegible] new and [illegible] scientific body.

Annual General Meetings will be held and arrangements are in hand for the next meeting in Utrecht in 1968.

The American [illegible] Association announces its plans to sponsor an [illegible] Congress on the subject of Child [illegible] 1968 at the Sheraton [illegible] Hotel [illegible] Houston. [illegible] further information [illegible] Congress may be obtained by [illegible] Ph.D. Chief [illegible] Research Center [illegible] University [illegible] Pittsburgh [illegible] Hall, [illegible] U.S.A.

Acta Pædiat Scand 57 177–185 1968

REFLEXES AND THEIR RELATIONSHIP TO BEHAVIOURAL STATE IN THE NEWBORN

H.-G. Lenard, H. von Bernuth and H. F. R. Prechtl

From the Department of Developmental Neurology, University Hospital, Groningen, The Netherlands

At one time the quantitative assessment of responsivity in the neurological examination of young infants seemed impossible owing to the variability of many reflex patterns. Since few systematic studies had been carried out the clinical impression was one of unpredictable changes in intensity and even in form of response patterns. Gradually however it became apparent that responsivity of reflex mechanisms is dependent upon behavioural state (36, 39). Knowledge of the relationship between state and responsivity thus became an essential prerequisite in designing a standardized neurological examination for newborn infants.

An attempt to design such a standardized examination procedure, taking account of state as a major variable, was made several years ago in this laboratory (38). Recommendations were made as to the optimal states and non-optimal states for eliciting particular reflexes, the intensity of each reflex being allocated a position on an ordinal scale. The optimal states suggested for elicitation of each reflex were based not upon detailed experimental analyses, but were derived inductively from clinical examination. A more detailed experimental analysis between certain reflexes and behavioural state was therefore required. In a pilot study 30 infants had previously been examined. As a result of this pilot study a selection of reflexes of different modalities was made for incorporation in the main study.

Such a study would have two purposes, one practical and one theoretical. The practical aim would be to throw more light on reflex responsivity in states other than the optimal one, since in paediatric practice, it is not always feasible to examine the baby in its optimal states. The theoretical aim would be to use the variable, behavioural state, as a tool in the further elucidation of the neurophysiological organization of reflex mechanisms in the human neonate.

SAMPLE AND METHOD

20 infants, aged 4–8 days, were selected from the nursery of the obstetric department. All these infants were full-term and of normal birth, following an uncomplicated pregnancy and delivery. Paediatric examination and neurological screening test did not reveal any abnormalities. Each baby was observed and tested for 2–3 hours between two feeds whilst lying undressed on a bassinette in a climate chamber (32–33°C, 50–60% humidity).

The reflexes were tested in random order. Each reflex was elicited once in a particular state and, if possible, a second time when the same state occurred again. The first reflex of the series was only tested when the baby had been in a stable state for at least 4 minutes. The interval for the elicitation of the different reflexes was never shorter than half a minute which assured the detection of a possible change of state.

For the technique of elicitation of the reflexes, scoring, and criteria for the states we followed our previous method (38). Reflexes were elicited in three states only:

State I (regular sleep) Eyes closed, no eye movements, regular respiration, no body movements except startles.

State II (irregular sleep) Eyes closed, slow and rapid eye movements, irregular respiration, no gross movements, occasional muscular twitches.

State III (quiet alertness) Eyes open, no gross movements.

Reflexes were additionally examined whilst the baby sucked glucose from a bottle (marked "S" in the tables).

The technique of elicitation and the scoring will be given separately for each reflex together with the results.

TABLES

1 *Quadriceps reflex*

	Score −	+	++	+++
State I	0	6	26	5
II	18	17	2	0
III	0	20	14	0
S	0	9	17	1

2. *Biceps reflex*

	Score −	+	++	+++
State I	0	1	29	1
II	10	21	3	0
III	0	18	17	0
S	0	8	19	0

3. *Ankle clonus*

	−	+	++	+++
I	0	12	23	4
II	31	5	1	0
III	17	15	2	0
S	6	16	5	0

4 *Moro reflex*

	−	+	++
I	0	7	24
II	25	5	1
III	0	6	23

5. *Palmar grasp*

	−	+	++	+++
I	27	7	0	0
II	5	10	18	0
III	0	0	32	2
S	0	0	12	16

6. *Plantar grasp*

	−	+	++	+++
I	29	8	0	0
II	5	22	10	0
III	1	4	27	2
S	3	6	21	1

7 *Palmo-mental reflex*

	−	+	++
I	36	0	0
II	12	16	7
III	3	13	16

8. *Babkin reflex*

	−	+	++
I	35	0	0
II	11	18	5
III	1	9	22

9. *Glabella reflex*

	−	+	++	+++
I	16	20	3	0
II	0	1	33	3
III	0	0	29	3
S	0	4	24	1

10. *Lip-tap reflex*

	−	+	++	+++
I	17	15	2	0
II	1	10	19	4
III	0	17	14	3

11. *Skin reflex from thigh*

	−	+	++	+++
I	13	11	15	0
II	7	10	15	6
III	2	9	25	1
S	6	14	12	0

12. *Abdominal skin reflex*

	−	+	++	+++
I	2	5	29	3
II	1	9	25	3
III	0	7	27	2
S	5	10	18	0

13. *Babinski reflex*

	−	+	++	+++
I	0	6	30	0
II	0	3	32	1
III	0	1	28	2
S	0	2	26	0

14. *Magnet response*

	−	+	++	+++
I	32	1	0	0
II	30	1	0	0
III	26	5	1	0
S	10	14	3	0

PROCEDURE, SCORING AND RESULTS

1 *Quadriceps Reflex and Biceps Reflex*

Changes in the intensity of various tendon reflexes during sleep have frequently been observed. Kleitman (18) gives an extensive review of these investigations. In an earlier study from our laboratory (41) we have shown, using electromyographic criteria, that the knee jerk is best obtained during regular sleep and wakefulness whilst it is inconsistent and, if present, weak in irregular sleep

Procedure

Quadriceps reflex and biceps reflex were elicited by a tap with the examiner finger on the tendon. The stimulus was repeated up to three times if the reflex was negative in order to exclude false negative responses caused by incorrect localisation f the stimulus.

Scores

−	No response.
+	Just discernible response.
+ +	Good response.
+ + +	Very strong response, with at least a few clonic beats.

Results (Tables 1 and 2)

The results are in line with the earlier electromyographic findings for the quadriceps reflex and are identical for the biceps reflex. Responses were equally good in regular sleep and during wakefulness. Both reflexes were negative or weak in irregular sleep During sucking both reflexes tend to be stronger than during quiet wakefulness. This finding is not surprising since during sucking there is usually tonic activity in the extensor muscles of the legs and tonic flexor activity in the arms.

2. *Ankle Clonus*

A very active stretch reflex from the m. triceps surae, a sustained ankle clonus, is usually regarded as a sign of neurological impairment (16, 38). It has been assumed that the different states of the baby do not influence the presence or absence of the ankle clonus (38) Sherman *et al* (48) found a positive reflex response in half of all new born babies they investigated.

Procedure

Both thumbs were pressed against the anterio part of the infant's footsoles. The feet were then dorsiflexed with rapid, abrupt movement.

Scores

- – No clonus.
- + 1 clonic beats.
- ++ 3 clonic beats or more
- +++ Inexhaustible clonus.

Results (Table 3)

During regular sleep a positive reaction could be found in all infants. Occasionally we even observed an inexhaustible clonus in this state. In irregular sleep the ankle clonus is mostly absent. During wakefulness there was an equal number of positive and negative responses, the positive responses being mostly weak. Positive reflexes occur more frequently than negative ones during sucking.

3 *The Moro Reflex*

Since its first description in 1918 (28), the Moro reflex has been one of the most widely used tools in the neurological examination of the young infant. Mitchell (27) has given a review of the historical, clinical and neurophysiological aspects of this reflex. Though Rosenbaum (46) reported negative responses in ∠5% of all newborns, most investigators agree that the reflex is present in all healthy newborn infants. It has been mentioned that the Moro reflex elicited by the original method—hitting the surface of the bed on both sides of the baby's head—could be obtained in sleeping infants whilst the reflex elicited by head-drop could not (37). Differences in different sleep states have not yet been reported. Since we could not lift the baby without disturbing its state, w could use the original method only

Procedure

The examiner hit the surface on which the baby was lying on both sides of the baby' head at about 15 cm distance

Scores

- – No response
- + Only slight flexion in the elbows and slight abduction of the arms
- ++ Full response with, in succession, flexion at the elbows, abduction at the shoulders, extension at the elbows, and adduction at the shoulders

Result (Table 4)

The Moro reflex, elicited by the original method, was obtained during regular sleep and during wakefulness with the same frequency and intensity. During irregular sleep the reflex was usually absent weak responses were occasionally seen at the beginning or towards the end of phase of irregular sleep. During sucking the eflex was not elicited because of the danger of aspiration. In spite of the strong motor reaction the reflex does not disturb the steady state of regular sleep

4 *Palmar and Planter G asp Reflex*

The palmar grasp was first described in 1891 by Robinson (45) as clinging reflex Van Woerkum (51) detected the plantar grasp Several studies have shown that both reflexes are regularly present in healthy newborn infants (7 34 39 49) It is known that the palmar grasp is increased during sucking (14 35).

Procedure

The palmar grasp was elicited by inserting finger from the ulnar side into the baby hand and pressing the palmar surface. The plantar grasp was obtained by pressing the thumbs against the balls of the infant foot soles.

Scores

- – No response.
- + Weak and unsustained response
- ++ Good, sustained response.
- +++ Very strong, prolonged response th the infant' finger or toe-tips going white

Results (Tables 5 and 6)

In regular sleep both reflexes were mostly absent occasionally a weak and unsustained response was seen. In irregular sleep positive reflex responses were significantly more frequent (χ^2 calculated for presence or absence of a response, was 28.0· $p < 0.005$ for the palmar grasp and 31.0· $p <$ 0.005 for the plantar grasp). The increase of the palmar grasp during sucking as compared with quiet wakefulnes was also significant (χ^2 with Yates correction = 14.8 p 0.005).

5 *The Palmo-mental Reflex*

This reflex, first described by Marinesco & Radovici (26) is normally present in healthy full-term newborns (25) Parmelee (31) also found it to be positive in premature babies. H noticed a dependence of the eflex response on the behavioural state the response being slightly weaker in sleeping babies Reis (42) egards this reflex as primitiv or incomplete form of generalized somatic esponse to nociceptiv stimuli.

Procedure

The reflex was elicited by scratching the baby hypothenar with a thumb nail.

Scores

- No response.
+ Just visible response.
++ Clearly visible muscle contraction with lifting f the chin.

Results (Table 7)

There was never a positive response in regular sleep. During quiet wakefulness the reflex response is stronger than in irregular sleep (χ^2 with Yates correction = 8.65 $0.025 > p > 0.01$).

6. The Babkin Reflex

This reflex—described in 1953 by Babkin in a Russian publication—has been thoroughly investigated in newborns by Lippmann (23). He found a positive response in 278 of 311 full term babies. Premature babies showed very strong responses, a finding later confirmed by Parmelee (32). Parmelee saw less positive responses in sleeping babies. He did not however at that time differentiate between different sleep states.

Procedure

The examiner applied rather firm pressure with his thumbs on the palms of the baby's hands.

Scores

- No response.
+ Mouth opening only
++ Mouth opening and head turning or head lifting.

Results (Table 8)

No positive responses were obtained during regular sleep. In irregular sleep the responses were significantly weaker than during wakefulness (χ^2 with Yates correction = 21.1 $p < 0.005$). The elicitation of this reflex frequently disturbed regular sleep or initiated gross movements if the baby was in irregular sleep or was awake.

7. The Glabella Reflex

Mechanical (9 21) or electrical (10 47) stimulation of the m. orbicularis oculi results in a reflex pattern which consists of two different reflexes: a first monosynaptic component, compatible with a myotatic reflex and observable only in EMG recordings and a second longer-lasting polysynaptic component with a longer latency which is easily observed clinically as a tonic contraction of the eye lid. According to Kugelberg (21) the afferent paths of both reflexes are to be found in the trigeminal nerve. The possible contribution of the facial nerve has been discussed by Rushworth (47)

Procedure

The reflex was obtained by short tap with the finger upon the glabella. The response observed was only the polysynaptic component of the glabella reflex.

Scores

- No response.
+ Just discernible response.
++ Clearly visible response.
+++ Sustained contraction of the lid for more than one second.

Results (Table 9)

The reflex was absent or weak in regular sleep. No differences could be found between the reflex responses in irregular sleep, quiet wakefulness and during sucking.

8. The Lip-tap Reflex

Like the glabella reflex the lip-tap reflex consists of a monosynaptic and a polysynaptic component. In a polygraphic study from our laboratory (40) it has been shown that the monosynaptic component (the lip-jerk) is larger in regular sleep whilst the polysynaptic *component (the lip-protrusion)* is more prominent during irregular sleep

Procedure

The reflex was elicited by sharp but gentle tap with the examiner finger upon the baby's philtrum. Only the lip-protrusion was observed and scored.

Scores

- No response
+ Short and weak protrusion of the lips.
++ Good response with clearly visible protrusion.
+++ Sustained protrusion for more than one second.

Results (Table 10)

In regular sleep the response was absent or weak. The frequency of positive responses was almost equal in irregular sleep and quiet wakefulness. Thus the clinically obtained results are the same as in the polygraphic study

9. *Exteroceptive Skin Reflex from the Thigh*

Peiper (33) mentions tonic skin reflexes to the limbs. Vlach (50) has investigated this group of reflexes in more detail. He has called them exteroceptive skin reflexes. Tactile stimulation of the skin overlying various muscles or muscle groups results in contraction of these muscles with or without corresponding movements. In a polygraphic study (41) it was shown that an exteroceptive skin reflex—dorsiflexion of the foot elicited by stimulation of the skin on the anterior surface of the ankle joint—is weak or absent in regular sleep and distinctly present during irregular sleep. In the present study a different site of stimulation was chosen.

Procedure

The skin of the lateral surface of the thigh was stroked gently with a round-tipped plastic probe. If the first response was negative, the stimulation was repeated and the response to the second stimulation scored.

Scores

- – No response.
- \+ Visible contraction of the muscle without corresponding movement.
- \+ + Abduction of the stimulated leg.
- \+ + + Generalized movement of the stimulated leg or both legs.

Results (Table 11)

No significant differences were found between the reflex responses in the different states. There was a tendency for stronger responses during wakefulness compared with sleeping and with sucking. This finding is in contrast to our earlier polygraphic study (41). This may be due to the different site of stimulation, or to differences in criteria—in the present case behavioural changes, in the earlier study electromyographic activity. The stimulation markedly alters the state of the infant.

10. *The Abdominal Skin Reflex*

According to various authors (1, 8, 13, 30, 46) the abdominal skin reflex (ASR) may be occasionally absent in newborn infants. Harlem & Lönnum (15), however, showed in an extensive and elaborate study on 200 infants that this reflex can always be obtained in healthy newborns if some patience and skill are applied in eliciting the reflex. The ASR is regarded as a spinal reflex with protective function (22). In infants its latency is longer than in older children and it fatigues more readily (24).

Procedure

The four quadrants of the abdominal wall were stimulated once successively by scratching lightly with a pin from the side towards the centre of the abdomen.

Scores

- – No response.
- \+ Just visible contraction.
- \+ + Clearly visible contraction.
- \+ + + Contraction of the abdominal wall and flexion on the ipsilateral leg in the hip joint.

Results (Table 12)

In all three states a positive response was obtained almost regularly. None of the infants showed an ASR constantly absent in all three states. As one would expect there was a tendency to weaker responses during sucking when the abdominal muscles were strongly contracted. Statistically, however, this difference was not significant (χ^2 with Yates correction = 4.74, $0.1 > p > 0.05$).

11. *The Babinski Reflex*

Stimulation of the foot-sole in infants causes a variable response pattern. 7 different patterns have been summarized (43, 44), ranging from flexion to extension of one or several toes. These patterns can be reduced to two, if the stimulus is applied in direction from the toes to the heel in order to avoid the plantar grasp reflex. One may obtain, then, dorsiflexion of the big toe or dorsiflexion of several toes, usually with spreading of the toes. The Babinski reflex of the newborn is very likely a different phenomenon from the Babinski sign encountered in pyramidal lesions of children and adults. As already suggested by Peiper (33) and Dietrich (7), lacking myelinisation of the pyramidal tract does not explain the presence and the variable features of the Babinski reflex in newborns. The reflex should be regarded rather as part of a triple flexion reflex of the whole leg—the picture Babinski reported in his original description in 1896 (2).

Procedure

The lateral side of the baby's foot-sole was stroked with thumb nail, from the toes to the heel.

Scores

- – No response.
- \+ Weak dorsal flexion of the big toe or more toes with or without spreading of the toes.
- \+ + Good dorsal flexion of the big toe or several toes with marked spreading of the toes.

Results (*Table 13*)

The reflex was obtained regularly and independently of the state of the baby A negative response was never obtained.

12. *Magnet Response*

Exact knowledge about the neurophysiological mechanisms underlying the magnet response in newborn babies is still sparse. In its appearance it has certain features in common with Sherrington's extensor thrust and with the magnet or exteroceptive supporting reaction described by Magnus. But since the latter reflexes were found in spinal or cerebellectomized dogs, comparison with the magnet response in newborn babies is difficult. Balduzzi (6) found a positive magnet response in 20% of newborns in both prone and supine positions. Lifting the baby to an angle of 45 increased the percentage of positive responses.

Procedure

Light pressure was exerted on the soles of the baby's feet with both thumbs. The examiner's hand maintained light contact with the feet when the legs extended.

Scores

- – No response.
- \+ Weak, incomplete extension.
- \+ + Sustained extension.
- \+ + + Rapid and long sustained extension.

Results (*Table 14*)

There were almost no positive responses in either sleep state. During wakefulness without gross spontaneous motor activity babies in supine position showed a positive response only occasionally More positive responses were obtained during sucking, probably favoured by the tonic extension activity in the lower limbs.

DISCUSSION

When considered in relation to different states of the infant, the reflexes studied fell into three groups.

In the first group are reflexes which are equally strong during regular sleep and wakefulness, but are weak or absent during irregular sleep. These are the knee jerk, the biceps jerk, the ankle clonus, and the Moro reflex. All these are *proprioceptive reflexes*. The depression of monosynaptic reflexes during irregular sleep is known already from polygraphic studies of the knee jerk (41) and the phasic component of the lip-tap reflex (40). Corresponding findings are reported from experiments in cats (3 12, 19) Depression of fusimotor function during irregular sleep (11, 20) as well as presynaptic inhibition of spinal and brain stem afferents (4 5) probably account for this phenomenon.

The three monosynaptic reflexes in our study show a tendency to increase when the baby is sucking. This trend might be due to the fact that during sucking the legs of the baby are tonically extended and the arms kept in tonic flexion. This may produce an increase in gamma-driving, in alpha-driving or both in these muscle groups and could be responsible for the stronger reflex responses.

It is surprising that the Moro reflex shows the same relationship to state as the monosynaptic reflexes. The proprioceptive nature of the reflex may account for this behaviour Since it is very likely that the centripetal pathway of the Moro reflex goes via the vestibular system one might hypothesize that presynaptic inhibition of the vestibular nuclei is responsible for the depression of the Moro reflex in this state. It remains necessary to investigate also the Moro reflex obtained by other techniques in different behavioural states.

The second group is formed by those responses which were mostly absent during regular sleep, weak during irregular sleep and strongest during wakefulness. All these were *exteroceptive reflexes* namely the palmar and the plantar grasp, the Babkin reflex, the palmo-mental reflex, and the tonic components of the glabella reflex and the lip-tap reflex. This finding is in accordance with our previous polygraphic studies of the lip-tap reflex (40). It is, however in contrast to results obtained in animal experiments (1.) where an electrically induced polysynaptic flexion reflex has been studied.

The reflexes in this group did not change during sucking, with the exception of the palmar grasp reflex, which did show a significant increase. This phenomenon has already been described (14 35) It is probably part of the increased flexor activity in the upper limbs during sucking.

In the third group are those reflexes (the abdominal skin reflex, the Babinski reflex, and the exteroceptive skin reflex from the thigh) which

did not show any alterations, but were easily obtained in all three states. These reflexes are *nociceptive reflexes*. The nociceptive character of the abdominal skin reflex has been stressed by Kugelberg & Hagbarth (22). The Babinski reflex of the infant may be regarded as part of a withdrawal reaction to a nociceptive stimulus. The exteroceptive skin reflex from the thigh is elicited by tickling, a low intensity form of pain stimulation. It seems reasonable that protective reflexes should function independently of the behavioural state. In the light of these results Reis (42) suggestion that the palmo-mental reflex is part of a generalized reaction to nociceptive stimuli, seems rather unlikely.

During sucking the ASR and the exteroceptive skin reflex from the thigh were sometimes slightly weaker. The contraction of the abdominal muscles and the tonic activity of the extensor muscles in the lower limbs during sucking probably account for this finding.

The magnet response does not fit into any of these groups. It was mostly absent in all three states. Positive responses can generally be elicited during active wakefulness or during crying but these states were not included in this study. Sucking increases the probability of obtaining a positive response. This again can be explained by the tonic extension of the legs during sucking.

Our findings give support to the notion that regular and irregular sleep do not represent different depths of sleep, but are two qualitatively different behavioural states (17, 29). The disappearance of exteroceptive reflexes in regular sleep does not mean that it is "deeper" than irregular sleep since monosynaptic reflexes are actually enhanced. These differences reflect special properties of the neurophysiological organization of these states. Conversely, the fact that regular sleep is very easily interrupted by nociceptive stimulation does not characterize this state as "light" sleep. It is rather a highly organized behavioural state whose homeostasis is difficult to regain once it has been disturbed. Nociceptive stimuli interfere also with other states, causing gross movements and irregularities in heart rate and respiration. These vegetative changes, however, can only be analyzed in detail by means of polygraphic techniques.

The tendon reflexes and even the Moro reflex, elicited by the original method, did not interfere with the state of the baby, nor did the exteroceptive reflexes with the exception of the Babkin reflex. For elicitation of the latter the palms of the baby's hands have to be pressed rather firmly which may give sometimes unpleasant or painful stimuli to the baby.

The following conclusions of this study may be of clinical significance: Proprioceptive and exteroceptive reflexes undergo marked changes in intensity during the behavioural states investigated. Reflex responses to nociceptive stimuli appear to be relatively independent of the behavioural state. Occasionally it may be difficult to keep a newborn infant awake during the course of a full neurological examination. It may fall into irregular sleep. Thus it may not be possible to continue the examination, because a condition is required wherein the reflexes are well expressed in order to assess pattern and symmetry.

Not unfrequently only a few neurological items are tested in the course of a general paediatric examination. In these instances it is easy to find absent, weak or definitely exaggerated reflexes. While these phenomena are abnormal signs in babies who are in the optimal state, this study has shown that they do occur in fact as normal physiological phenomena in non-optimal states.

Sucking may increase or inhibit certain reflexes. This fact must be regarded carefully if a nipple is used to pacify the infant during the examination.

SUMMARY

In 20 healthy newborn infants a series of 14 reflexes of various modalities was studied in different behavioural states (regular sleep, irregular sleep, quiet wakefulness) and during sucking.

According to our results these reflexes fall into three categories. Proprioceptive reflexes (quadriceps reflex, biceps reflex, ankle clonus and Moro reflex) show good responses during regular sleep and wakefulness, but are absent or markedly diminished in irregular sleep. Exteroceptive reflexes (plantar and palmar grasp, the Babkin reflex, the palmo-mental reflex, glabella reflex, and lip-tap reflex) are absent or diminished in regular sleep. During irregular sleep these reflexes can be elicited almost as well as during wakefulness. Nociceptive reflexes (abdominal skin reflex, the Babinski reflex and an exteroceptive skin reflex from the thigh) are equally present in the three states

investigated. The magnet response could not be obtained in any of the three states.

During sucking, the palmar grasp and the tendon reflexes tend to be stronger whilst the abdominal skin reflex and the exteroceptive skin reflex from the thigh are slightly weaker

The implications for the neurophysiological organization of different behavioural states in human neonates are discussed together with their consequences for the clinical neurological examination of the newborn infant.

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STUDIES OF URINARY TRACT INFECTIONS IN INFANCY AND CHILDHOOD

X. *Short or Long-term Treatment in Girls with First or Second-time Urinary Tract Infections Uncomplicated by Obstructive Urological Abnormalities*

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Investigations into the many problems met with in urinary tract infections (UTI) in childhood are often performed on clinical material comprising obstructed and nonobstructed (simple uncomplicated) cases of UTI, first-time and recurrent infections occurring in males and females, newborn and adolescents. It is the belief of the authors of this paper that many of the controversial opinions about UTI have their origin in experience gained from the use of such heterogeneous material. Furthermore the experience gained from one group of patients has often been extended to perhaps incomparable, groups. The prevail- predilection for long-term treatment of UTI affords a good example of this.

Several investigations concerning children published during the last 10–15 years have suggested that long-term therapy can reduce the frequency of recurrence better than short-term treatment (c.f. 7 8 18) Such investigations have been concerned with patients who had already manifested their tendency for repeated infections. By analogy it has been more and more accepted that long term treatment is also favourable in first-time infections, but no investigations have been directed to test the validity of this assumption.

Two investigations, that of Pryles *et al* (15) and that of Normand & Smellie (13) mark a considerable advancement in our approach to the study of urinary tract infections, since they attempted to choose clean material for their investigations on recurrence rate and the effect of therapy Thus they studied patients with first-time infections without complicating urological abnormalities. However both groups studied the effect of therapy only after treating their patients for long-term, six weeks and one year respectively and no controls were used. Thus it is still an open question whether short or long-term treatment should be recommended in first-time infections.

In this paper we will present our experience with short and longterm sulfonamide treatment in girls aged months–16 years, with first-time infections (or second-time infections in 1/7 of the material) uncomplicated by obstructive urological abnormalities. The study has shown that the recurrence frequency was very high and that it was not diminished by extending the sulfonamide administration for 2 months. In addition the investigation has presented some characteristic features of first-time infection.

MATERIAL AND METHODS

The material is derived from patients who during the years 1959–1964 consulted our department because of acute urinary tract symptoms. Details of selection and methods will be given below but in short the study is concerned with 279 girls aged 2 months–16 years, who appeared with their first or second "uncomplicated" UTI, Table 1. Patients with obstruction of the urinary flow stone formation or radiographically demonstrable renal parenchymal reduction have thus been excluded. The patients selected for the study were hospitalized for two weeks and randomly divided into two groups, one treated for 10 days the other for 60 days. Prescheduled

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Table 1 *Past history of urinary tract infections before entering the study*

	Girls
Probable first-infections	237
One infection previously	42
Total	279

check-up studies were performed during the first year after the original infection.

History. A careful history was taken to reveal previous UTI. Children with more than one previous infection, diagnosed or even much suspected, were excluded.

Age and sex. Girls under two months of age and boys of any age were not included in this study because it is the authors belief that those categories may constitute special problem. The age distribution of the material appears in Fig. 1.

Diagnosis. All urine samples were voided and collected after careful cleansing of the external genitalia (23). Thus no recurrences could be attributed to catheterization. The samples were immediately cooled on ice and within couple of hours cultured by quantitative method (23).

A bacterial count of 100,000 colonies per ml or more has been taken as evidence of *UTI*. However, patients with counts between 10,000 and 500,000 together with doubtful other diagnostic criteria have, if possible, been reexamined before definite decision was made to include them in the study. Bacterial sensitivity tests were performed on peptone-free placenta infusion agar according to the single disc method of Ericsson *et al.* (5).

Urological investigation. During the period covered by this study the indications for intravenous urography and micturition urethrocystography in girls over the age of two months have, in brief, been as follows: (1) One or more recurrent infections. (2) First-time infection in which fever did not subside or in which urine was not cleared of bacteria or pus cells within couple of days after the beginning of therapy. (3) First-time infections with slow normalization or failure to return to normal of the concentrating capacity or the *E. coli* antibody titre. These indications for urological investigation will probably reduce considerably the risk of overlooking patients with obstruction, stone formation or more marked renal parenchymal reduction. Thus, although intravenous pyelography has been performed only in 60 per cent of our patients, can assume that the material consists only of simple infections.

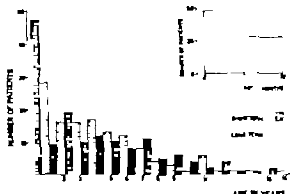


Fig. 1. Age distribution of the patients in the two therapy groups. In the right upper corner graph is put in showing the youngest age group subdivided into two age groups not further divided into therapy groups. There is predominance of younger patients even after the group below one year of age.

The urinary white cell excretion as determined on uncentrifuged urine, the cells being counted in Fuchs-Rosenthal chamber. A count of more than 10 cells per mm³ was regarded as pathological. Determination of the renal concentrating capacity (22) was regularly performed at the time of the initial infection and in about half of the reexaminations and was then of diagnostic aid in some instances, as was the *E. coli* antibody titre (1).

Therapy. Sulfisoxazole (Gantrisin®) was administered either for short term (10 days) or a long term (2 months). The doses given appear in Figure 2. The patients were randomly selected, even birth date numbers were chosen for long-term therapy and the others for short-term therapy. We have not investigated systematically the extent to which given instructions were followed. However good personal contact and a knowledge of the timing for new prescriptions make us believe that the above classification is realistic.

Follow up. The patients were checked at about 13, 30, 60, 90 and 365 days after the first day of therapy. Every investigation included quantitative bacterial colony and white cell count. In half of the instances the patients were hospitalized for 24 hours during the reexaminations. Treatment was stopped at least 60 hours before urine cultures were performed, Fig. We judge this to be necessary routine, even when shortacting sulfonamides as sulfisoxazole are used, if the colony count is to be relied upon (24). Table 2 shows to what extent the patients



Fig. 2. Diagram showing treatment periods, sulfonamide doses and prescheduled check-up. During the initial 10-day treatment sulfonamide was administered in four daily doses, after the 13th day in a morning and an evening dose.

Table 2. *Participation rate at each check up given in per cent of total number of patients sheduled for investigation*

Patients with recurrences are excluded from the time of their recurrence

Check up at about ...	13	30	60	90	365 days
On short-term therapy	100	97	90	83	88*
On long-term therapy	100	100	94	85	93*

One patient in the short-term group and two patients in the long-term group did not come to any examination after cessation of therapy Of the patients not appearing t the one year check up ⅔ have been examined later

presented themselves to the scheduled reexaminations. In addition the patients were examined whenever they displayed symptoms suggestive f UTI. Medical advice was easily obtainable without any cost to the patients in this study Thus both symptomatic and asymptomatic recurrences have been detected.

The figures on recurrence rate refer to the *number of patients* with recurrence and not to the number of recurrent infections in the whole material.

RESULTS

A. Comparison of the two treatment groups with regard to the frequency of recurrence

The frequency of recurrence about 60 hours after finishing the initial 10 day course of therapy was ... to 4 per cent (11 patients). Two months ... it had risen to 18 per cent and one year after the original infection the cumulated recurrence rate was 36 per cent. There was no significant difference in the overall recurrence rate between the two treatment groups as indicated in Figs. 3 and 4

Grouping of the material in three age categories: 0-1 year 1-4 years and 4-16 years or division with regard to the clinical type of the original infection, i.e. febrile or nonfebrile, did not reveal any differences in recurrence rate between the two treatment groups.

B. Comparison of the two treatment groups with regard to the clinical type of recurrence

A difference between the groups was established already at two months (that is at the moment of cessation of the long-term therapy) when the cumulative frequency of asymptomatic and symptomatic recurrences was 8 and 10 per cent respectively in the short-term group compared to 13 and 5 per cent respectively in the long-term group, Fig. 3 and Fig. 4 The difference between the two therapy groups is statistically significant ($0.01 < p < 0.05$). This predominance of asymptomatic recurrences in the long-term group remained essentially unchanged during the following 10 months.

C. Analysis of bacterial etiology and drug resistance in the original infections and in the recurrences

The results are summarized in Table 3 As can be seen the original infections were comparable in the two treatment groups with regard to bacterial etiology and sulfonamide sensitivity Notable is the rarity of simultaneous infection with two bacterial strains. Coliform bacteria dominated and caused 9/10 of the original infections. Likewise 9/10 of these original infections were caused by sulfonamide sensitive strains.

Looking at the recurrences some marked differences between the treatment groups show up,

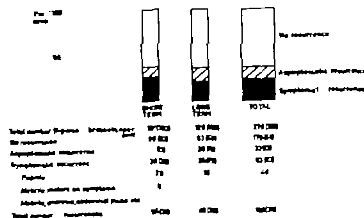


Fig 3 Recurrence rate during the first year following an acute infection in 279 girls ... nonobstructive, predominantly first-time urinary tract infections Each patient with ... recurrence is represented only once. One third of the patients had recurrence In this study there were no better results ... long-term than ... short-term treatment.

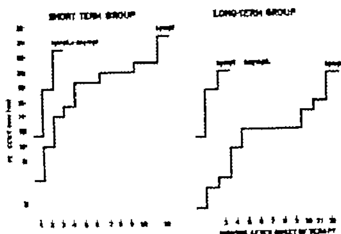


Fig. 4. Cumulative frequency of patients with symptomatic and asymptomatic recurrences in short and long-term group during first year after original infection. Each patient is represented only once. In the short-term group the symptomatic recurrences occurred early: more than 50 per cent during the first of the observation period. The same probably holds true for asymptomatic infections in both short and long-term group: see text.

especially during the first two months, that is during the time the long-term group received sulfonamide. Resistance to this drug was then observed in 17/29 (59 per cent) of the patients belonging to the short-term group and in 21/22 (95 per cent) of the patients of the long-term group. This difference is statistically highly significant ($p < 0.01$). It can be added that out of 11 recurrences diagnosed at the 13 day check up (that is immediately after closing the first period of therapy) all were resistant to sulfonamide. Further details of these very early recurrences are presented below. These figures as well as others given in Table 3 may suggest that sulfonamide administration favours the appearance of sulfonamide resistant bacteria at the recurrences, in particular when the interval between the last period of treatment and the recurrence is short.

The *in vitro* sensitivity to nitrofurantoin and ampicillin of sulfonamide-resistant strains isolated at an original infection or at a recurrence is presented in Table 4. All strains except one were sensitive to nitrofurantoin. Sensitivity to ampicillin was in this small material somewhat less frequent.

D. *Time lapse between initial infection and first recurrence*

This question is partly elucidated by Figures 4 and 5. Figure 4 shows that in the short-term group more than 50 per cent of the symptomatic recurrences occurred within 3 months after the original infection, that is during 1/4 of the observation time. In the long-term group the corresponding figure was only 25 per cent. In this group there was, however, an accumulation of early asymptomatic recurrences, so that the total recurrence rate at three months was about the same in the two groups.

The discovery of asymptomatic recurrences was limited to the five scheduled examinations. Four of these took place during the first three

Table 3. *Infecting organisms and their sensitivity to sulfonamide in the original infection and in recurrences*

Figures are given in per cent of the number of patients in each respective group

Group	Original infection		Recurrences, 0–60 days		Recurrence 61–365 days	
	Short-term	Long-term	Short-term	Long-term	Short-term	Long-term
Coliform bacteria	89	93	83	68	79	90
Other bacteria	10	5	14	23	17	5
Mixed infections	1	2	3	9	3	5
Sulfonamide-sensitive bacteria	90	90	41	5	70	58
Sulfonamide-resistant bacteria	10	10	59	95	30	4
Absolute number of patients in each respective group	131	1[illegible]8	30	22	25	23

Table 4 *The sensitivity to nitrofurantoin and ampicillin of sulfonamide-resistant bacteria*
Bacterial strains isolated at original infections and recurrences

Type of bacteria	N of strains		No. of strains sensitive to Nitrofurantoin		Ampicillin	
Coliform bacteria	57		57/57		31/40	
Other bacteria	22		21/22		9/10	
Enterococci		17		17/17		8/8
Klebsiella		2		2/2		1/1
Proteus		1		1/1		0/1
Pseudomonas		1		0/1		—
Lactobacillus		1		1/1		—
Total	79		78/79		40/50	

months. Therefore the frequency of early and late asymptomatic recurrences cannot be compared in the same way as the symptomatic ones.

However Fig. 5 shows that in both treatment groups the number of asymptomatic infections is higher at the first two examinations (13 and 30 days) than at the two following ones (60 and 90 days). This difference becomes significant ($p < 0.05$) when long and short-term groups are put together. When comparing these figures it should also be remembered that the first two examinations cover a period of one month while the latter two cover a period of two months. This lends support to the opinion that asymptomatic recurrences, like the symptomatic ones, are apt to occur shortly after the original infection rather than later.

Eight of the eleven early recurrences at the 13-day check-up were analyzed to elucidate the question of whether a persistence of the original infection was present or if a new invading bacterial strain was responsible. This was done by aid of serological typing as described earlier (2). In six of the eight infections there was a change of bacteria indicating that a new infection had appeared immediately after cessation of therapy. Table 5.

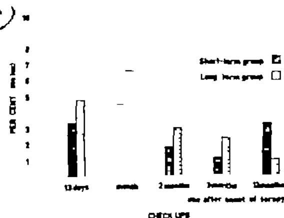


Fig. 5. Prevalence of asymptomatic recurrences at five consecutive prescheduled examinations following the original infection. Each patient with recurrence is represented only once. The frequency of recurrence at each check-up is figured out on the basis of the number of patients, who had not yet had a recurrence.

The figure suggests that asymptomatic recurrences are more likely to show up early after the original infection rather than late.

Table 5 *Bacterial typing in eight patients with recurrence 2½ days after completing the 10 days course of therapy for the original infection*

The results show change f bacteria which in 6 of the 8 patients indicates reinfection. I one of the tw patients without change of bacteria, the original infection was sulfonamide-resistant. All recurrences were sulfonamide-resistant.

^{r} = resistant to sulfonamide, 01 = belonging to *E. coli* 0 group 1. *E. coli* = belonging to *E. coli* but 0-group not determined, *Ent* = enterococci; *Klebs* = Klebsiella

Patients	Bacteria isolated at original *infection*	Bacteria isolated 2½ days after completion *of therapy*
E. S.	0 1	0 1
I. A.	0 15	0 11
N. H.	0 1	*Klebs*^{r}
L. T.	*E. coli*	*Ent*^{r}
M. H.	0 2	0 2
E. K.	0 2	0 33
C. L.	*E. coli*	*Ent*^{r}
L. F.	*E. coli*	*Ent*^{r}

E. Frequency of over-all ecurrence rate in dif ferent age groups irrespective of therapy

When the patients were divided into three age groups 0-1 year 1-4 years and 4 16 years the recurrence rate was found to be respectively .7 39 and 40 per cent. By chi-square calculation the difference was found to be close to significance at the 5 per cent level. That this difference mainly depends on the youngest age group becomes more evident if we calculate the confidence limits of the expected frequency of recurrence in each respective group [P $(0.26 < p < 0.28) = 0.95$ $P_2(0.37 < p < 0.41) = 0.95$ $P_3(0.39 < p < 0.41) = 0.95$].

DISCUSSION

This investigation is concerned with 79 girls with their first-time, or in a few cases, their second-time UTI. All infections were simple that is uncomplicated by obstruction, stone formation or marked radiographically demonstrable parenchymal defect. It is shown that the frequency of cure after a 10 day course of sulfonamide was 96 per cent when cultures were taken about 60 hours after therapy was finished, 82 per cent months later and 64 per cent after one year These figures show how cautious one has to be before classifying a patient with UTI as cured. This was also recently pointed out by Freedman *et al* (6) and by McCabe & Jackson (11) studying adults. These two investigations as well as our show that eradication of the actual infection is not equivalent to a cure of the disease just as the interruption of an acute asthmatic attack is not the same as cure of atopy The high recurrence rate, probably due to some predisposing factor(s), is a strong motive not to consider a patient as cured until long-term follow up over a period of years has shown him to be free of infection and, if possible, progressive renal scarring.

An important item of our study was that the frequency of recurrence was the same whether the patients got prophylactic sulfonamide two months after the initial therapy or not. This statement seems to hold true even if the follow-up is extended beyond one year A preliminary work-up of the material 3 /₂ years after the original infection shows a cure rate of 57 per cent (3) as compared to 64 per cent after one year in both treatment groups.

Since the frequency of recurrence the same both with and without prophylaxis t means that the patient on prophylactic treatme t being free of infection during one year or even 3 ears, would have been so also without proph laxis. This should not be taken as a general argument against long-term prophylaxis. The lue of such a regimen seems to have been demons ated in patients with an established tendency for ec rence e.g. Stansfeld & Webb (18) and Normand & Smellie (13). Our study only suggests that girl with a first-time infection and without malformations will not benefit from prophylaxis, at least not from prophylaxis with sulfonamide when this agent was used for therapy The possibility of using other drugs will be discussed below

The high frequency of recurrence, and the influence of therapy on recurrence rate must be discussed and evaluated in relation to earlier published studies. There is no material completely matching ours but the closest comparable ones seem to be that of Pryles, et al (15) and that of Normand & Smellie (13). The former author re ported on 41 children, boys included, with their first infection and an initial period of treatment (sulfonamide or nitrofurantoin) of 6 weeks. Their recurrence rate was 24 per cent during a follow up period of 1 34 months and compares well with ours. This investigation also supports our finding that extending sulfonamide therapy beyond 10 days has no influence upon recurrence rate. Markedly dissimilar are the figures obtained by Normand & Smellie (13). In 31 children (boys included) with first-time infection covered by sulfonamide for an average time of a full year following the acute infection they found only one recurrence during therapy These authors explain their good results as a consequence of the long term prophylaxis and ecommend that it be used in all first-time infections The discrepancy between their results and ours may partly be explained on the basis of difference in methods and in composition of material. Thus their study group was composed of 1 3 boys and 2/3 girls and the mean age was low Both these factors male sex and age below one year—may be associated with a somewhat lower recurrence rate as indicated by the present study and forthcoming one (4) Another and probably more important difference is that in their study urine cultures wer performed while the patients were on

ACKNOWLEDGEMENTS

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THE VENOUS PLASMA FREE AMINO ACID LEVELS OF MOTHER AND CHILD DURING DELIVERY

II. *After Short Gestation and Gestation Complicated by Hypertension with Special Reference to the "Small for Dates" Syndrome*

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The ratio between total free amino acid levels in cord and maternal plasma is decreased in pregnancy complicated by toxaemia (12, 14 10, 6). Since the incidence of pregnancy toxaemia is high among mothers of "small-for-dates" infants (13 1), i.e. infants with a lower birth weight than expected according to gestational age, the question arises if the change in the ratio of amino acid levels is of significance to the disturbed foetal growth. To elucidate this problem the individual amino acid levels of cord and maternal plasma, especially those of the essential amino acids, have been estimated during delivery in cases with hypertension. The results have been compared to the levels of uncomplicated pregnancies ending at (27) or before term. Out of the known maternal conditions associated with the "small-for-dates" syndrome hypertension during pregnancy has been chosen in this study

In order to give a background to the material which has been used, a study concerning the relation between hypertension during pregnancy and foetal weight and length as compared to non-hypertensive cases with the same period of gestation has been made. This survey has also been made in order to demonstrate if the material studied is representative with regard to the problem to be elucidated

This work was made possible through grant from AB Semper Fund f Nutritional Research

Hypertensive disorder of pregnancy and ... and length at birth

The incidence of pregnancy ... of delivery Intrauterine and perinatal ... well as weight and length of the infant ... relation to gestational age was ... material of 9023 consecutive deliv... stetrical departments in Stockholm ... period January 1964 July 19... the clinical classification were as fol...

Pregnancy toxaemia (T) ... pressure above 140/90 mm Hg ... two determinations, proteinur... crease of more than ... of ... weight.

Preeclamptic toxaemia ... headache in addition to ...

Eclamptic toxaemia (E) ...

If the period of gesta... lished or if pregnancy ... fest diabetes the case... for length of gestation

The data are pre... vidual birth weight been plotted out Figs. 1 and 2. ... nancies compli... out the other hypertension when no ...

Table 1 *Number mother s age percentage of primigravidae percentage of children with birth weight below −2 S.D for gestational age intrauterine and perinatal mortality in different groups of hypertensive disorders during pregnancy*

	No.	%	Mother's age, years (mean)	Primigravidae (%)	Birth weight < −2 S.D. (%)	Praelabour deaths, no.	Perinatal deaths, no
Toxaemia	75	0.83	26	69	24	1	1
Praeclampsia	18	0.20	27	72	28	1	1
Eclampsia	3	0.03	30	(66)	(33)	0	0
Total	96	1 10	26	70	25	2 = 2.1 %	2 = 2.1 %
Hypertension	55	0.60	27	58	16	1	2
Essential hypertension	14	0.15	31	50	14	0	1
Total	165	1.85	27	61	21	3 = 1.8 %	5 = 3.0

The total number of pregnancies was 9023

oped (Hess) are also presented in Table 1 As can be seen from the table all the different forms of hypertensive disorder seem to predispose to the birth of small-for-dates infants. In accordance with what has been demonstrated earlier (8) the highest incidence of "small-for-dates babies was found in cases of pregnancy toxaemia, particularly when there was a spontaneous delivery before term (?) The increased mortality is well documented (32, 20)

AMINO ACID STUDIES

Methods

The methods, including collection of samples, deproteinization and ion-exchange-chromatography were identical to those described earlier (27). Through a change of the temperature 25°C for 100 minutes, 55°C fo an addi-

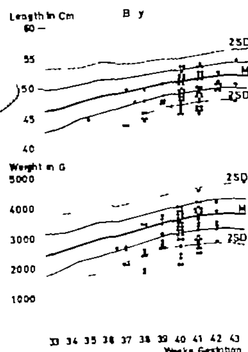


Fig. 1 Pregnancy toxaemia. The individual birth weights and crown-heel lengths have been plotted onto the Swedish standard (15). Both sexes have been plotted onto the boys standard.

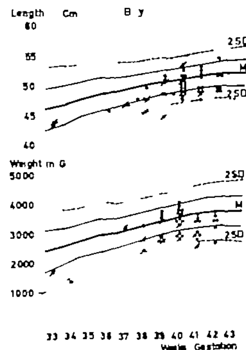


Fig 2 Hypertension during pregnancy For explanation see Fig. 1 Dot with line means the cases where hypertension was known before pregnancy

Table 2. *Short gestational period*

Age, weight before pregnancy, height and number of pregnancy of the mothers. Length of gestational period and weight, crown-heel length and sex of the child at birth

Mother				Child			
Age (years)	Weight (kg)	Height (cm)	No. of pregnancy	Gestation (weeks)	Weight (g)	Length (cm)	Sex
21	45	164	I	34	2120	46	Boy
25	53	162	II	36	2200	45	Boy
33	58	164	I	34	2340	44	Boy
36	56	159	II	33	1830	43	Boy
23	51	166	II	35	2210	45	Girl
25	55	170	III	37	2500	48	Girl

tional 15 minutes and then 25°C again, it was possible to separate threonine and serine from glutamine and asparagine in some of the samples by an additional run.

Material

During delivery plasma from the mother cubital vein and the cord vein was studied in the following groups.

I. *Short gestational period.* Six cases. The mother' age, weight before pregnancy height, the number of the pregnancy and the gestational period are shown in Table 2. The mother' weight and height before pregnancy were within standard deviation for age (7), except for one mother whose weight as 6 kg below −1 S.D. and another mother whose height was 2 cm above +1 S.D. There was no history of previous disease except for previous short pregnancies in 3 of the mothers. All had crown vaginal delivery the mothers blood pressure during pregnancy and labour was less than 140/90 and there was no proteinuria or glucosuria. The maximum total time of labour was 10 hours. The anaesthesia used was nitrous oxide for one hour before delivery in 4 cases, and 4–6 g of chloroform during delivery in 2 cases. There was no sign of perinatal asphyxia. All the newborns had an Apgar score of 10 points at one, and four minutes after delivery. In no instance was the cord around the neck or otherwise obstructed. As can be seen from Table 2 all the children had birth weight and crown-heel length within 2 S.D. for gestational age and sex (15). All babies were discharged healthy without signs of malformations from an uneventful neonatal period except for moderate hyperbilirubinaemia in 3 infants. In one case the placenta showed large infarction, but otherwise it was found to be normal. The placental weight was within standard deviation for gestational period (22) except for the infarcted one, which was slightly less than −1 S.D.

II. *Hypertensive disorder.* Eight cases. The mother' clinical classification, age, the number of the pregnancy and the gestational age, birth weight, length and sex of the child are shown in Table 3 and Fig. 3. In the case of one with preeclampsia and low foetal weight and length, and of another of pregnancy toxaemia with normal foetal weight and length caesarean sections were performed. Otherwise there were normal crown vaginal deliveries. In no case was there perinatal asphyxia, nor was the cord around the neck or otherwise obstructed. Maximum time of labour was 6 hours. In the vaginal deliveries nitrous oxide was given as anaesthesia in one case and 3 g of chloroform in another case. Glucose was given intravenously to 2 mothers before delivery. In one of the cases the baby had normal birth weight and length, in the other one weight and length was low for gestational age. In the first case Nepresolme®, Pethidine® and Hibernal® were also given intravenously. In the PE case the urinary oestriol excretion in the mother had been below 10 mg/day for 3 weeks before delivery and the child showed low birth weight for gestational age.

In 4 cases the birth weight and length were below −1 S.D. for gestational age. These cases will be presented separately. Three of them had birth weight length below −2 S.D. None of the infants developed neonatal hyperbilirubinaemia. One of the babies with normal birth weight showed jitteriness and hypoglucosaemia. Since the intravenous glucose tolerance test showed an abnormally high rate of glucose clearance this baby obviously had

Table 3. *Hypertensive disorder during pregnancy*

Clinical classification, age and number of pregnancy of the mothers. Length of gestational period and weight, crown-heel length and sex of the child at birth

For symbols see text (hypertensive disorder of pregnancy).
* below 1 S.D. for gestational age
** below 2 S.D. for gestational age

Mother			Child			
Clinical classification	Age (years)	No. of pregnancy	Gestation (weeks)	Weight (g)	Length (cm)	Sex
PE	34	I	36	1870*	45	Boy
T	30	I	41	3450	49	Girl
H	[illegible]	II	41	3060	52	Boy
T	33	IV	39	2630	48	Boy
Hess	39	III	38	2810	45	Boy
T	21	I	40	3120	50	Boy
T	41	V	37	2030	43	Girl
H	27	V	39	3400	49	Boy

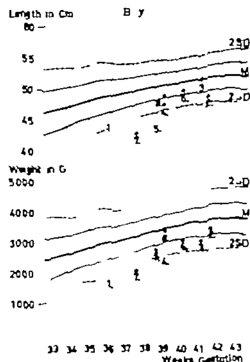


Fig. 3. The eight cases of hypertensive disorder during pregnancy in which plasma amino acids were determined. For explanation see Fig. 1.

neonatal hypoglycaemia. Another of the small-for-dates babies showing jitteriness had normal blood glucose level. In no case were any malformations diagnosed and the infants were all discharged healthy after an uneventful period. The placental weights for 3 of the 4 cases with low weight and length at birth were below 2 S.D. for gestational period (22).

RESULTS

Short gestational period. The ratio between the cord and maternal levels of taurine and aspartic acid showed a great standard deviation due to occasional high levels in the cord plasma (Fig. 4). The mother's venous plasma showed an increase of glutamic acid and the cord vein plasma an increase of methionine only. The data are presented in Table 4.

Hypertensive disorder. The data are presented in Table 5. In addition to changes typical of a short gestational period there was an increase of methionine in the plasma of the mother. The standard deviation of urea was high due to marked variations in the levels of the mothers. In cord plasma there was an elevated concentration of glutamic acid, aspartic acid and urea.

Hypertensive disorder with an offspring with low foetal weight and length for gestational age. As can be seen from Table 6 there was in these 4 cases, in addition to changes typical of the short gestational group and the whole hypertensive group, a decrease in the ratio between the cord and maternal plasma in the levels of valine, isoleucine, leucine, lysine, phenylalanine, methionine, tyrosine and urea. The differences were statistically significant ($1\% < p \leq 5\%$) with respect to valine, methionine, tyrosine and urea. The ratio of the alanine levels were not significantly increased (Fig. 5). The low ratios of the methionine levels were also seen in the 8 cases complicated by

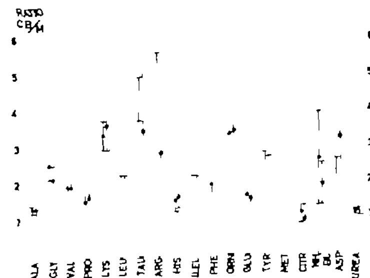


Fig. 4. Mean ± double standard error of the ratios of the cord vein amino acid level to that of the mother's cubital vein. Cases with short gestational period (dotted lines) are compared to normal cases (solid lines).

Table 4. *Short gestational period*

Free amino acid levels in venous plasma from the mother during delivery and the cord vein during delivery with ratios in between the cord vein level and that of the mother's cubital vein

Range, mean and standard deviation are expressed in μmol/l plasma. n = number of cases where the actual amino acid was separated in such a way that an accurate estimation was possible

	Mothers				Cord-blood				Ratio, cord/maternal plasma			
Amino acid	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	
α-Alanine	220–548	6	362	145	344–535	6	426	68	0.8–1.7	6	1.3	0.4
Glycine	79–134	6	101	25	156–234	6	201	31	1.4–2.7	6	2.1	0.5
Valine	88–235	6	140	61	210–[illegible]84	5	241	35	1.1–2.4	5	1.9	0.5
Threonine	72–313	3	171	126	210–353	4	264	63	1.1–3.0	3	1.9	1.0
Proline	64–162	6	102	34	126–161	6	148	10	0.9–2.0	6	1.6	0.4
Serine	53–103	3	72	27	109–135	3	125	13	1.3–2.3	3	1.9	0.5
Lysine	69–179	6	111	37	282–49[illegible]	6	377	79	2.0–4.5	6	3.6	0.9
Leucine	41–124	6	72	30	87–129	6	108	14	0.9–2.5	6	1.7	0.6
Taurine	42–107	6	67	24	106–408	6	188	111	1.3–8.4	6	3.4	2.8
Arginine	22–32	3	27	5	67–74	6	71	3	2.3–3.4	3	2.8	0.5
Histidine	62–129	6	85	24	110–140	6	126	9	0.9–1.9	6	1.6	0.4
Isoleucine	22–41	6	37	15	44–64	6	55	6	0.9–2.3	6	1.7	0.6
Phenylalanine	28–59	6	41	13	66–77	5	72	5	1.1–2.6	5	2.0	0.6
Ornithine	20–43	5	27	9	65–98	6	82	14	2.1–4.4	5	3.4	1.1
Glutamic acid	43–95	6	66	20	76–180	6	92	57	0.5–3.6	6	1.5	1.1
Tyrosine	19–51	6	33	14	40–93	6	65	18	1.3–2.9	6	2.2	0.6
Methionine	10–41	6	20	11	20–41	6	34	8	1.0–2.9	6	1.8	0.6
Citrulline	5–28	6	12	8	5–14	6	9	3	0.5–1.8	6	0.9	0.5
α-NH_2-BU	3–17	6	10	4	9–21	5	15	5	1.0–2.8	5	1.9	0.7
Aspartic acid	4–37	4	15	15	5–47	5	18	17	1.0–7.2	3	3.2	3.5
Urea	1535–4335	6	3171	1025	1823–4243	6	3214	927	0.9–1.[illegible]	6	1.1	0.1

Table 5. *Hypertensive disorder during pregnancy*

For explanation see Table 4

	Mothers				Cord-blood				Ratio, cord/maternal plasma			
Amino acid	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.
α-Alanine	160–470	6	327	123	223–798	8	545	181	1.1–2.6	6	1.6	0.5
Glycine	89–169	6	137	35	196–405	8	307	73	1.2–2.4	6	1.9	0.4
Valine	98–180	6	150	31	174–338	8	237	62	1.2–2.1	6	1.7	0.4
Threonine	110–186	2	148	—	187–534	5	323	—	1.6–1.7	2	1.7	—
Proline	77–123	6	101	19	97–283	8	184	58	1.3–2.3	6	1.8	0.3
Serine	51–87	2	69	—	87–185	3	140	—	1.7	2	1.7	—
Lysine	76–141	6	112	26	228–463	8	340	69	2.4–4.3	6	3.2	0.7
Leucine	53–98	6	82	16	91–175	8	125	32	1.2–2.3	6	1.6	0.4
Taurine	34–145	6	71	49	134–591	8	297	154	1.7–16.9	6	6.6	6.3
Arginine	20–53	3	34	17	19–71	7	48	51	0.9–3.6	2	2.3	1.9
Histidine	72–112	6	97	16	102–184	8	131	76	0.9–1.6	6	1.4	0.2
Isoleucine	32–59	6	47	9	49–104	8	73	17	1.3–[illegible]5	6	1.8	0.4
Phenylalanine	35–49	6	41	4	55–86	8	67	12	1.5–2.1	6	1.6	0.2
Ornithine	23–45	6	33	9	42–178	8	112	42	2.2–7.1	6	3.5	1.8
Glutamic acid	64–114	6	86	23	56–360	8	162	98	0.7–5.5	6	2.2	1.9
Tyrosine	27–39	6	32	5	46–85	8	62	15	1.5–2.7	6	2.0	0.5
Methionine	9–30	5	22	8	21–49	8	37	11	1.0–2.4	5	1.6	0.5
Citrulline	7–20	5	12	5	8–15	8	11	3	0.7–2.1	5	1.1	0.6
α-NH_2-BU	10–21	4	14	5	1[illegible]–33	8	18	7	1.0–1.7	4	1.4	0.3
Aspartic acid	4–22	5	10	8	22–58		38	14	1.4–14.5	5	6.4	5.8
Urea	2900–8350	6	4240	2066	[illegible]900–7140	8	4550	1196	0.9–1.2	6	1.0	0.1

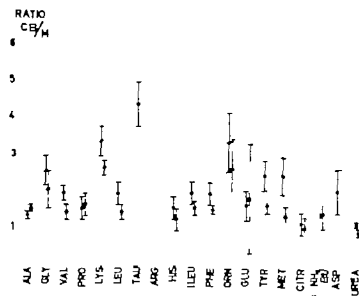


Fig 5 Mean ± double standard error of the ratios of the cord vein amino acid level to *that of the mother's cubital vein.* Cases with hypertensive disorder during pregnancy and low birth weight and crown-heel length for gestational age of the infant (dotted lines) are compared to normal cases (solid lines). The ratios of taurine and aspartic acid levels showed a marked standard error as in the cases with short gestational period.

hypertensive disorder In the mother's venous plasma there was a significant increase of valine, isoleucine and leucine (Fig. 6) ($0.1\% < p \leq 1\%$) and of lysine, histidine and urea ($1\% < p \leq 5\%$) The cord vein plasma levels showed no differ ences from those found in the "short gestation group" or in the "hypertensive group

DISCUSSION

In the perinatal mortality survey of the National Birthday Trust of 1958 in Britain, the post mortem findings were mainly those caused by anoxia in more than half of the stillbirths (9) Since preeclamptic toxaemia was one of the main maternal complications in those cases this condi-

Table 6. *Hypertensive disorder during pregnancy and low birth weight and crown-heel length for gestational age he child*

explanation see Table 4

	Mothers				Cord-blood				Ratio, cord/maternal plasma			
Amino acid	Range		Mean	s.D.	Range		Mean	s.D.	Range		Mean	s.D.
Alanine	160–398	3	256	126	223–756	4	480	238	1.4–1.6	3	1.5	0.1
Glycine	105–169	3	146	36	196–405	4	312	95	1.7 2.4	3	2.0	0.4
Valine	145–180	3	166	18	178–264	4	225	36	1.2 1.5	3	1.4	0.2
Threonine	—		—	—	246–358	2	302	—	—	-	—	—
Proline	77 112	3	93	18	97–233	4	170	60	1.3–1.8	3	1.6	0.3
Serine	—		—	—	185	1	185	—	—	-	—	—
Lysine	95–141	3	123	25	228–388	4	330	71	2.4–2.8	3	2.6	0.2
Leucine	82 98	3	91	8	97 151	4	170	26	1.2 1.5	3	1.4	0.2
Taurine	34–145	3	74	62	134–453	4	288	135	1.7 12.1	3	5.9	5.5
Arginine	20–29	2	25	6	19–71	3	47	26	3.6	1	3.6	—
Histidine	82 111	3	99	16	102 134	4	120	17	0.9–1.4	3	1.2	0.3
Isoleucine	44–59	3	52	8	64–81	4	74	8	1.3–1.7	3	1.5	0.2
Phenylalanine	38–49	3	42	7	56–78	4	67	10	1.5–1.6	3	1.5	0.1
Ornithine	28–41	3	35	7	62 147	4	107	42	2.[illegible] 3.4	3	2.6	0.7
Glutamic acid	67 114	3	91	23	62 213	4	199	69	0.7–3.2	3	1.8	1.3
Tyrosine	29–39	3	33	5	46–77	4	59	13	1.5–1.8	3	1.6	0.2
Methionine	22 30	3	25	4	21–49	4	38	14	1.0–1.6	3	1.3	0.3
Citrulline	9–20	3	13	6	8–14	4	12	3	0.7–1.3	3	1.0	0.3
NH_2-BU	10–21	2	16	8	12 33	4	20	10	1.[illegible] 1.6	2	1.4	0.3
Aspartic acid	5–22	3	11	10	25–52	4	34	12	1.4–10.4	3	5.3	4.6
Urea	3160–8345	3	4932	2950	2982 7140	4	4957	2082	0.9–1.0	3	0.9	0.1

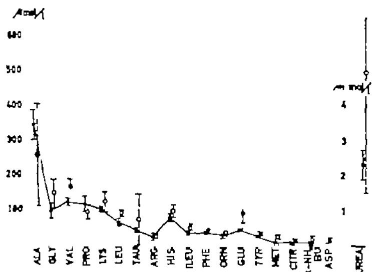


Fig. 6 Mean ± double standard error of the individual amino acid levels plus urea level. The mothers with hypertensive disorder during pregnancy where the offspring as of low birth weight and crown-heel length for gestational age (dotted lines) re compared to normal mothers (solid lines)

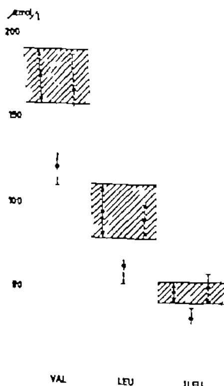


Fig 7 Mean ± double standard error of the branch-chained amino acid levels in normal non-pregnant women (dots and solid bars and lined area), in normal pregnant women (dots and dotted lines) and in mothers with hypertensive disorder during pregnancy where the child was of low birth weight and crown-heel length for gestational age (rings and dotted lines)

tion evidently gives rise to a failure of oxygen transfer from the mother to the foetus. Another evidence of disturbed transport from mother to foetus in toxaemia is the finding of a diminished sodium transport from mother to foetus (16, 11 23). A possible background for these findings is that uterine and placental blood flow is consider ably decreased when pregnancy is complicated by toxaemia (5 1 30, 38).

The high incidence of low birth weight for gestational age in pregnancy toxaemia (Table 1) may depend upon a uteroplacental malabsorption with the consequent insufficient supply to the foetus of those amino acids being essential. Therefore it might be of interest to see whether there are at birth in this condition any changes in the ratio between cord and maternal plasma amino acid levels.

The deviation from the normal conditions found in the cases with short gestational period is mainly that of an occasional exaggeration of the normally high level of taurine in the cord plasma. To a lesser extent the same seems to apply to lysine (Table 4). The explanation might be lower uptake or metabolism by the foetal liver as has already been proposed with regard to the relatively high levels at term (27). The levels of glutamic acid and aspartic acid may have been increased by the hydrolysis of glutamine and asparagine during storage or processing of the sample. The cord plasma level of methionine is

normally very low. The elevation of the total cord amino acid level in cases of short pregnancy (26, 18, 37) might be the result of the very high taurine level.

The "hypertensive disorder group" seems to be characterized mainly by an increase of the urea level in the cord plasma. This has also been found in "acute foetal distress" (28) and in dysmature babies (35). The concentration of urea has been found to be elevated in amniotic fluid in preeclamptic cases with prolonged foetal distress (36). In the present study the mother's urea level was occasionally found to be very high but the mean for the individual ratios between the cord and maternal levels was 1.0, i.e. the normal one. Thus, it seems likely that the high cord and amniotic fluid levels are only secondary to occasional high plasma levels in toxaemic mothers.

In the cases of hypertensive disorder with low foetal weight and length for gestational age the ratios between cord and maternal plasma in the levels of all the essential amino acids studied were found to be low. The same was found for tyrosine, which amino acid, however, has to be regarded as an essential amino acid for the foetus in view of the lack of phenylalanine hydroxylase activity in the liver of foetuses (25). The finding of a lower ratio of the phenylalanine level is in dance with what has been reported earlier. The significantly higher level in the mother's of the three essential amino acids, valine, isoleucine and leucine, while the levels in the cord plasma were the same as in uncomplicated pregnancies, indicates that the lower ratio of the essential amino acid levels is the consequence of a higher level in the mother. In fact, it has already been demonstrated from studies of the level of α-amino-nitrogen (29, 14, 10) and from studies by means of paper chromatography (3, 39) that the total amino acid level is higher in toxaemic pregnancies than in normal ones. If the plasma levels of branch-chained amino acids of these mothers is compared to the levels of nonpregnant women, *it is evident that the normal decrease* during pregnancy has not taken place (Fig. 7). It may be that the high levels of the branch-chained amino acids in toxaemic pregnancies reflects a decreased transport to the foetus. The facts that the α-amino-nitrogen concentration in maternal plasma rises to nonpregnant level when the umbilical cord is obstructed (10) and in one or two days after delivery (4) are in accordance with such an assumption.

The low maternal plasma levels as found in uncomplicated pregnancies may be governed by some factor, probably a hormonal one. In this connection, oestrogen has to be considered, since its anabolic effect is well-known. There is evidence for oestrogen production by the human placenta (34). In vitro the amino acid uptake of placental cells is enhanced by oestradiol (33). Furthermore, in studies on rats it has been demonstrated that oestrogen stimulates RNA and protein synthesis in the uterus *in vivo* (31, 19). A drop in urinary oestriol excretion in the mother has been correlated to "foeto-placental insufficiency" (24, 17). In our material the excretion was low in at least one case. Normal amino acid levels in cord plasma do not contradict an assumption of a decrease in the foetal supply of all or some of the essential amino acids since other factors may be responsible for a normal plasma amino acid homeostasis in the foetus.

SUMMARY

In cases of hypertensive disorder there is an increase of urea in the cord vein plasma. The increase seems to be secondary to an occasional increase in the maternal plasma.

In cases of hypertensive disorder during pregnancy associated with intrauterine growth retardation of the foetus the ratio between the cord vein plasma levels and the mother's cubital vein plasma levels of essential amino acids is lower than under normal conditions. The low ratios of valine, isoleucine and leucine levels are shown to be due to higher maternal plasma levels at delivery than in normal pregnancies. It is suggested that the findings have some bearing on the growth retardation of the foetus, being the consequence of a diminished supply of essential amino acids to the foetus.

The differences from what is found in normal term pregnancies are not due to a shortening of the gestational period, since there is in the foetus, in a gestational period down to 33 weeks, only still higher plasma levels of taurine and lysine.

To demonstrate that the material is representative as regards hypertension and the "small-for dates" syndrome a survey has been made of the weight and length at birth in cases of hypertensive disorder during pregnancy.

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Table 1 *Hemostatic studies in patients with glycogen storage disease Type I*

Patient	Age (yrs.)	Sex	Bleeding tendency	Platelet count (× 10³ per mm³)	Ivy bleeding time (min)	Clot retraction (%)	Quick prothrombin (%)	P T T (sec)	Platelet adhesiveness (range in %)	ADP aggregation (sec)	Platelet factor 3 availability (sec)
T S.	20	M	Yes	580.0	> 15	50	70	40.0	17–22	13	50
K. H.	17	F	Yes	344 0	> 15	50	130	38 4	0 (3)	26	69
C. S.	14	F	Yes	340.0	> 15	60	130	36.0	8–26	25	—
K. W	13	M	Yes	336.0	> 15	50	100	32.5	6–16	13	44
G M	1 ½	M	Yes	318.0	> 15	60	130	32.1	1–18	11	27
V W	2	F	N	531 0	8–10½	60	120	31.6	5–71	8	26
D R.	1	F	No	512.0	8–13	50	130	39.0	0–3	9	26
S. B.	1½	M	No	334.0	8	—	—	—	19–32	8.4	27
Normal values				140–350	< 8	40–64	70–130	< 50	> 55	< 30	14–31

availability was determined using Russell's Viper Venom as outlined by Spaet & Cintron (17).

RESULTS

The pertinent clinical and laboratory data are summarized in Table 1 Five patients had a history of frequent spontaneous epistaxis and easy bruising but no life-threatening hemorrhage has occurred. Patients T.S., K.H., G.M and S.B had been subjected to open liver biopsy during the first few years of life without significant bleeding complications. In the five patients with a positive history the hemorrhagic tendency was generally not noted prior to five years of age. No hemorrhagic episodes have yet occurred in the three younger children.

Whole blood platelet counts were within the high normal range or above but no thrombocytopenia was documented in spite of repeated counts at various times. Platelet morphology appeared normal on routine smears and under phase microscopy Bone marrow aspirate was examined in one patient (T.S.) and the megakaryocytes were normal in number and appearance.

Marked prolongation of the Ivy bleeding time to beyond 15 minutes was found consistently in the five older patients. The three younger patients had bleeding times at the upper limit of the normal range for our laboratory and on one occasion it was prolonged to 13 minutes in patient D R. and to 10 / minutes in V W Clot retraction, partial thromboplastin times and prothrombin times were normal in the seven patients tested.

Three patients exhibited an abnormality in PF 3 availability but the Stypven time of the PRP was normalized after freezing and thawing the PRP Macroscopic aggregation of platelets following addition of ADP occurred within 30 seconds in all 8 patients.

Platelet adhesiveness was measured on at least three separate occasions in each patient. In 7 patients the proportion of platelets lost after contact with the glass microspherules was consistently and significantly reduced when compared to our normal controls. The range of values obtained in these 7 children is given in Table 1 the highest level being 3 % on one occasion in patient S.B. The only patient who failed to exhibit a consistent abnormality in platelet adhesiveness was V W who, on two occasions had adhesiveness in the normal range. The proportion of platelets lost by aggregation and adhesion was increased to above 80 % in all specimens when 0.1 ml of 10^{-3} M ADP was added to the glass microspherule system.

A transfusion of 6×10 platelets from 3 normal donors was administered to patient C.S. and her platelet count rose from 340 000 per mm³ to 45[illegible],000 per mm³ but platelet adhesiveness remained unchanged at 20%. Normal PRP with 69% adhesiveness was incubated for 30 minutes with an equal volume of platelet poor plasma from patient C.S. and this resulted in a reduction in adhesiveness of the normal platelets to 2[illegible]

In order to determine whether hypoglycemia or lacticacidosis played a role in the development of the platelet lesion, patient D R. received an intravenous infusion of glucose (2 g/kg) over a hour period. This produced a significant rise in blood

sugar and a marked decrease in serum lactic acid without altering platelet adhesiveness.

DISCUSSION

Abnormal platelet function in patients with GSD Type 1 provides an explanation for the hemorrhagic tendency reported in this disease. Although the clinical manifestations, markedly prolonged bleeding times and diminished PF 3 availability were present only in the older patients, the defect in platelet adhesiveness appears unrelated to age, since the youngest patients in our series also exhibited severe abnormalities. These findings tend to confirm the observations of Alagille *et al.* (1) who found that a definite bleeding tendency could not be documented until late into the second year of life. Frequent prolongation of the bleeding time, abnormalities in platelet thromboplastic activity and a slight but constant increase in whole blood platelet counts in their patients with GSD Type 1 were also observed. The elevated platelet counts are not, however in the thrombocythemic range found in patients with myeloproliferative disorders in whom defective platelet adhesiveness has been reported (14). Furthermore, dilution of our patients PRP with their own platelet-poor plasma to achieve platelet concentrations of 400,000 per mm³ did not alter the degree of adhesiveness.

Although studies were performed on only one patient, there is a suggestion that the abnormal platelet function may be related to the plasma environment since platelets from normal individuals appear to lose their adhesive properties when exposed to plasma from patient C.S both *in vivo* and *in vitro*. The failure to improve platelet adhesiveness after glucose infusion in patient D.R. *is similar* to an earlier observation by LeLong *et al* (1) who used the bleeding time as an index of platelet function.

The biochemical basis for the platelet lesion remains in doubt. Alagille and co-workers (1) reported decreased levels of platelet glucose-6-phosphatase in two of their patients but Ockerman (15) states that all the glucose-6-phosphatase activity in platelets is due to the action of nonspecific phosphatases. Several authors ha re-ported an increase in platelet glycogen in GSD Type 1 and this may play a role in altering platelet function (1 13).

The ability of platelets from patients with GSD Type 1 to adhere and aggregate normally after addition of ADP suggests that some extra- or intra-cellular factors may inhibit production or release of endogenous ADP Similarly their failure to release PF 3 may be dependent on an absence of available ADP (8) Patients with GSD Type 1 are known to manifest hyperuricemia and hy perlipemia (5 11) and these compounds may play a role in influencing platelet function by altering the state of the cell membrane. However it has been suggested that increased levels of free fatty acids are associated with an increase in platelet adhesiveness (10) and it appears that this, too is mediated by ADP released from platelets (9) In view of the fact that patients with GSD Type 1 have inordinately high levels of serum free fatty acids (6) it is possible that their action is limited in these patients by lack of available ADP Other lipid components are elevated in this condition but it is not known what effect they may have on platelet function.

The platelet lesion which we have described in GSD Type 1 bears some similarity to the abnormality reported in patients with uremia (3 16). Decreased platelet adhesiveness with normal ADP-induced aggregation and abnormalities in PF 3 release appear to play a role in the development of the bleeding tendency in uremia. It has also been reported that platelet function returns to normal following dialysis and renal transplantation suggesting that, as in GSD alterations in the plasma environment predispose to the platelet abnormality (3)

Abnormal platelet function, therefore, appears to provide the basis for the hemorrhagic tendency in patients with GSD Type 1 but, as in uremia, the exact nature of the defect has not been established and further studies of the various intra- and extracellular factors which may play a role in the development of the platelet lesion are currently being undertaken.

SUMMARY

Because of the ill-defined bleeding tendency which has been reported in patients with Glycogen Storage Disease, Type 1 various aspects of hemostasis and coagulation were studied in 8 patients. Definite abnormalities in the bleeding time and platelet factor 3 availability were found in the

older symptomatic individuals but all the patients, irrespective of age, exhibited significant reduction in platelet adhesiveness in spite of normal platelet aggregation after addition of ADP Although an intracellular defect may account for the abnormal platelet function, environmental plasma factors appear to play a role in the development of the platelet lesion.

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GLUCOSE INFUSION IN THE PREGNANT RABBIT AND ITS EFFECT ON GLYCOGEN CONTENT AND ACTIVITY OF FOETAL HEART UNDER ANOXIA

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During labour the foetal supply of oxygen through the placenta and the umbilical cord may be temporarily interfered with, causing the foetus to become more or less hypoxic. In the presence of advanced hypoxia, all the tissues of the foetus are dependent on the availability of carbohydrate substrate for energy metabolism. In anoxia, the central nervous system—which has no ppreciable carbohydrate store of its own—is completely dependent on the supply of glucose via the blood stream, which can take place only as long as the circulation is functioning. Consequently it should be of interest to study the prerequisites for maintenance of the circulation, i e., the heart s ability to contract.

It has been demonstrated that the ability of an isolated and anaerobic turtle heart to perform contractions is dependent on its glycogen content (7). It should therefore be possible to influence the duration of cardiac activity in the foetus during anoxia by changing the glycogen store of the heart.

In the monkey the heart rate of the foetus is less affected by anoxia *than is that of the new* born, which was suggested by Jacobson & Windle (5) to be due to the greater glycogen reserve of the foetal heart. This interpretation is compatible with the report of Dawes *et al.* (1) of a positive correlation between the time that foetuses of various species could withstand anoxia and the glycogen content of the foetal heart. This content depends on maternal nutrition. Thus, Shelley (8) stated that, in the guinea-pig, a 40% reduction of the glycogen content of the foetal heart could be brought about by starving the mother for 48 hours before delivery

The present study in rabbits was undertaken to ascertain whether

1 Giving the mother a glucose infusion prior to delivery would *increase* blood glucose and heart glycogen content in the foetus.

If so, whether

2. Any relation exists between these factors thus affected and the duration of maintained cardiac activity during anoxia.

Finally whether

3 Other foetal parameters, such as pH and liver glycogen content, would be affected by infusion of glucose in the mother

MATERIAL AND METHODS

Altogether 41 rabbits of local breed were studied on the 29th day of pregnancy e., *2 days before term* (Table I). T 23 rabbits with an average weight of 3940 g (range 3100–5000 g), an i. infusion of 30% glucose solution was given at rate of 20 ml/hour during 6 hours. Totally 120 ml (9 ± 0.3 g/kg body eight; mean + s.e.) were given. The hypertonic solution was delivered through small polyethylene catheter (PE 10) which, through needle puncture of an auricular vein, was advanced until reached the superior vena cava. The 18 controls with an average eight of 3840 g (range 3000–5100 g) were immobilized for 6 hours, as were the experimental animals. As rule, control was matched with an experimental animal, and the tw were studied concurrently

Within 10 minutes after the infusion, the animals were sacrificed by intracentral injection f 2 ml of 2% X locaine® solution. One member of the team handled

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A preliminary report of this study was presented at the Symposium on Foetal Distress in Siena, Italy September 16–18, 1966.

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Table 1 *Number of animals in which the various parameters were studied.*

Glucose-infused animals	
ECG	10
ECG blood glucose, heart and liver glycogen	8
ECG, pH	5
Total	23
Controls	
ECG	5
ECG, blood glucose, heart and liver glycogen	8
ECG, pH	5
Total	18



Fig. 1 Blood glucose concentration during anoxia in foetuses of glucose-infused mothers and in control foetuses (mean ± s.e.).

the experimental animal, and another member the control. *The uterine horns were exposed, and in each animal one* foetus was rapidly separated from its placenta, and transferred to a container with liquid paraffin at 37°C, from which the foetal ECG was recorded. The remaining foetuses in each litter were transferred with intact membranes to a bath of saline solution at 37°C. In this way each foetus was completely deprived of its oxygen supply. As long as heart activity could be recorded by ECG foetuses from the same litter were sacrificed at irregular intervals, whereby samples were obtained for biochemical analyses. In two foetuses, the heart activity had stopped before sacrifice, and they were excluded.

The pH of the blood was determined in five litters in each group. The sample was taken directly from the heart, after the apex of the ventricle had been removed for glycogen determination. Blood glucose was determined by the *o*-toluidine method (3). For determination of the *trichloroacetic acid (TCA)-soluble glycogen content, tissue* samples were taken from the apex of the heart, and from the anterior edge of the right liver lobe. Glycogen was determined according to Huijing (4). The pH of maternal and foetal blood was determined by the Astrup micro method (9). For practical reasons all parameters could not be studied in each animal. *In Table 1 it is* seen in how many animals the various examinations could be carried through.

RESULTS

Blood glucose. In the glucose-infused rabbits, the maternal blood glucose ranged from 114 to 488 mg/100 ml, whereas in the controls it ranged from 84 to 108 mg/100 ml. This difference ($p < 0.001$) was reflected in the first foetal values recorded, which were a mean 303 mg/100 ml (range 86–742 mg) in the experimental group and a mean 76 mg/100 ml (range 3[illegible]–94 mg) in the controls (Fig. 1). After 51–70 minutes, the mean value in the controls had risen to 203 mg/100 ml, this increase in the initial being highly significant ($p < 0.001$). In the foetuses of glucose-infused rabbits, on the other hand, the initial hyperglycaemia persisted essentially unchanged during the same period of anoxia.

Heart glycogen. The content was determined at various intervals during the period of anoxia. The mean of the initial values was [illegible]24 ± 0.12 g/100 g wet weight (mean ± s.e.) in the foetuses of glucose-infused mothers, and 1.56 ± 0.14 g/100 g wet weight in the foetuses of the controls. The difference (0.68 g) is highly significant ($p < 0.001$). During subsequent periods—i.e., 11–30, 31–50, 51–70 and 71–90 minutes—the difference persisted, and was significant except during *the interval 71–90 minutes (Fig. 2 a Table [illegible])*.

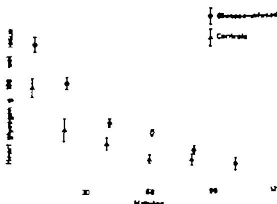


Fig. 2 a. Heart glycogen content in relation to duration of anoxia in both groups (mean ± s.e.).

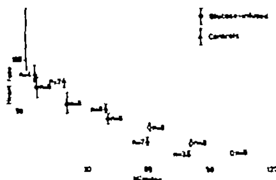


Fig. 2 *b*. Heart rate during anoxia (mean ± S.E.).

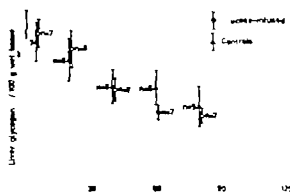


Fig. 3. Foetal liver glycogen during anoxia (mean ± S.E.).

Liver glycogen. The content was not significantly higher in the experimental foetuses than in the controls. During anoxia, it decreased at the same rate in the two groups (Fig. 3). The difference between the first and last values was significant in the experimental group ($p < 0.01$), whereas no significant decrease was demonstrable in the controls (Fig. 3).

Heart activity. This persisted for 92 ± 3 minutes (mean ± S.E.) in the foetuses of glucose-infused rabbits, but only for 67 ± 4 minutes in those of the controls (Fig. 4). The difference between the mean time of maintained heart activity in the two groups (25 minutes) is highly significant ($p < 0.001$).

Heart rate. During the period of anoxia, the heart rate decreased successively in both groups. During no interval was there any difference between them in this respect (Fig. 2 *b*).

Blood pH. The pH of the maternal blood ranged from 7.37 to 7.44 in the glucose-infused rabbits, and from 7.31 to 7.50 in the controls, i.e., it was within normal limits in both groups. On the other hand, as seen in Fig. 5, even the initial values in the foetuses disclosed acidosis, which increased with the duration of anoxia. It should nevertheless be noted that the acidosis was consistently of about the same degree in both experimental and control foetuses at the beginning of anoxia. The pH fell successively during the period of anoxia, it showed the same pattern in both groups. At no time was there any significant difference in pH. However, with a continued fall in pH, heart activity persisted longer in the experimental group. Consequently, the pH was significantly lower when the heart activity ceased in the experimental group than in the control group (mean 6.28 and 6.39, respectively; $p < 0.01$).

DISCUSSION

It has been shown in different species that a certain correlation exists between the initial glycogen content of the foetal heart and the ability to withstand asphyxia (1). It has been concluded from these studies that the glycogen content of the heart is, in fact, the decisive factor for the ability of the foetus to survive under anoxic conditions. These studies do not, however, provide conclusive

Table 2. *Foetal heart glycogen g/100 g wet tissue (mean ± S.E.) during anoxia*

Duration of anoxia (min) →	0–10	11–30	31–50	51–70	71–90	91–110
Glucose-infused Group I	2.24 ± 0.12 n = 8	1.62 ± 0.09 n = 7	0.98 ± 0.08 n = 8	0.83 ± 0.06 n = 8	0.5 ± 0.08 n = 7	0.32 ± 0.10 n = 5
Controls Group II	1.56 ± 0.14 n = 8	0.87 ± 0.17 8	0.64 ± 0.10 8	0.41 ± 0.07 7	0.39 ± 0.10 4	
Difference I–II	0.68	0.75	0.32	0.42	0.16	
p	0.001	0.001	0.05	0.001		

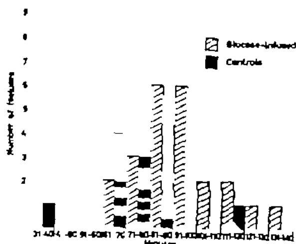


Fig 4 Grouping of the foetuses in relation to maintained heart activity

proof, since other factors—such as the body size and body temperature—may have played a role when different animal species were compared. For this reason, we made our studies on one species only and with fixed temperature. Passmore & Schlossmann (6) among others, have shown that during a glucose infusion, the blood glucose concentration in the foetus reflects that in the mother

Our experiments show that the heart glycogen does, in fact, is significantly higher in foetuses whose mothers have been given an infusion of glucose (about 9 g/kg body weight). Thus, the heart glycogen in foetuses of glucose-treated

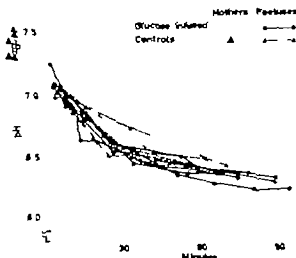


Fig 5 Blood pH in foetuses of glucose-infused and control mothers. The first value shows the maternal pH. On cessation of heart activity the pH was significantly lower in the treated group.

mothers was an average 40% higher than that in foetuses of untreated mothers, the difference between the groups being highly significant. On the other hand, no significant difference was present between the liver glycogen content in foetuses of glucose-infused mothers as compared to those of mothers not given glucose.

It has been demonstrated that the liver glycogen store of the rabbit foetus is low up to the last days of intrauterine life, when a marked rise occurs, concurrently with a decrease in the glycogen store of the placenta (2). Under our experimental conditions it was not, however possible to produce a further increase in the glycogen content of the foetal liver by infusing glucose in the mother on the 29th day of pregnancy This may be ascribed to the presumption that, at the end of pregnancy glycogen synthesis in the liver already at a normal blood glucose level is at a maximal rate.

During the period of anoxia, the heart glycogen fell in both groups at approximately the same rate, and when it fell to around 0.4 g/100 g wet weight, the ECG-recordable heart activity ceased. Thus, the foetuses of glucose-treated mothers—who initially had a higher heart glycogen level—showed a longer duration of heart activity This implies that, in asphyctic foetuses, the heart glycogen content appears to be an important factor for the duration of heart activity The glycogen utilization per time unit falls successively during the period of anoxia (parallel with falling heart rate) and ultimately ceases, despite the store not being completely empty The ECG-recordable heart activity ceases concurrently Since glycogen is presumably the only utilizable substrate for energy production in the heart under anoxia, the rate of breakdown should reflect the total energy production. The fact that the energy supply does not suffice for heart activity—even though the glycogen supply is not fully utilized—may be due to the energy production requiring a relatively high substrate content, or to the rate of glycogenolysis being arrested by enriched metabolites or finally to the metabolites formed inhibiting the course of contraction in the muscle in some other way

The pH of the blood fell in a similar way in both the control and the experimental group. This is reasonable, since the fall in pH should be caused mainly by an accumulation of lactic acid. Presumably this is largely formed in the heart

muscle, which is the only organ carrying out active work. The heart activity persisted for a longer time in the foetuses of glucose-treated mothers, which implies a continued production of lactic acid. This can explain the significantly lower pH of the blood on cessation of heart activity in this group.

Thus, on the average, the heart activity ceased at the same glycogen content in both groups, whereas the pH of the blood was significantly lower in the foetuses of the glucose-infused mothers. The degree of acidosis in the extracellular fluid does not, therefore, seem to be a decisive factor for the cessation of glycogenolysis. It must, however be pointed out that we did not have the possibility of determining the intracellular pH in the two groups.

In preliminary experiments, we observed a considerable accumulation of lactic acid in anoxic foetal hearts. In these experiments, we also noted a remarkable accumulation of potassium in the heart muscle, indicating that the intracellular lactic acid pool had been partly neutralized by the entry of potassium ions in exchange for hydrogen ions. Whether this potassium accumulation is, actually a factor of importance for the ability of the foetal heart to perform work under anoxic conditions is now being investigated.

In foetuses of glucose-infused mothers, the blood glucose concentration was relatively high, and underwent no significant change during the period of anoxia. In the controls, on the other hand, the blood glucose was initially lower (normoglycaemic). Glycogenolysis in the liver took place at the same rate in both groups, despite the rise in blood glucose being recorded only in the untreated group. This implies that the liver glycogen metabolism cannot explain the difference between the blood glucose response in the two groups. It must, however be emphasized that our experiments give no information about the extent to which the glycogen breakdown in the liver results only in glucose production, or whether the utilization also occurs in some other way

The pulse rate in the two groups had essentially the same course, i.e., it fell successively Here certain parallelism exists between the glycogen utilization per time unit and the pulse rate, implying that both glycogen utilization per time unit nd pulse rate are high initially and then fall successively

SUMMARY

The object of the present study was to determine the possibility of increasing the glycogen content of the foetal myocardium, and thus its ability to survive under anoxic conditions, by infusing glucose in the mother animal before delivery

The material consisted of 41 rabbits ..3 were given a glucose infusion, and 18 served as controls. On the 29th day of pregnancy a solution of 30 % glucose was infused over a 6-hour period. Immediately after the infusion, the mother was sacrificed and the foetuses delivered abdominally They were maintained at 37 C, but without the possibility of lung aeration The ECG of one foetus in each litter was recorded until no heart activity remained. Concurrently the other foetuses were sacrificed at irregular intervals, and studied with respect to the glycogen content of heart and liver blood pH and blood glucose. These parameters were correlated to foetal heart activity

It was found that glucose infusion in the mother did, in fact, increase the glycogen content of the foetal heart. This, in turn, seemed to increase the ability of the foetal heart to continue its activity under anoxic conditions.

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STUDIES ON ERYTHRO-KINETICS IN INFANCY

XI. *The Change in Circulating Red Cell Volume During the First Five Months of Life*

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During the first week of life the intense erythropoetic activity present at birth decreases to very low values. The low rate of red cell production persists for 1–2 months (9, 10, 12, 13) and is one factor which determines the decrease in the red cell values of the peripheral blood during early infancy. Estimations of the change in circulating red cell volume (RCV) during the first weeks of life have been made from serial measurements of plasma volume (PV). Simon *et al.* (21, 22) found a decrease of RCV whereas this was not the case in a study of Csorba & Jeszenszky (7). The decrease in RCV reported by Simon *et al.* (21, 22) is smaller than the decrease in the total haemoglobin mass calculated by Schulman & Smith (20) and Gairdner *et al.* (10), who based their calculations on serial measurements of peripheral blood values and body weight and prediction formulas of total blood volume.

Knowledge about the change in total haemoglobin mass or circulating red cell volume during early infancy plays an important part in evaluation of the erythrokinetics of this period. As earlier estimations, based on different indirect methods, are conflicting and as no direct measurements of RCV are available, such measurements were made on 22 full-term infants aged 0–138 days and 16 premature infants aged 0–98 days using ^{51}Cr-labelled autologous red blood cells. In 15 of the infants serial measurements were made with 2–3 determinations in each infant.

MATERIAL AND METHODS

All infants included in this study were normal (one infant had palatoschisis and one polydactylia) except for the prematurity of some of them. They were born in vaginal deliveries by healthy mothers. Their umbilical cords were clamped after cessation of the pulsations, which mostly occurred about 5 minutes after birth.

Sixty measurements of circulating red cell volume (RCV) were performed on 22 full-term infants aged 0–138 days and 16 premature infants aged 0–98 days, according to a method using ^{51}Cr-labelled autologous red blood cells described in detail earlier (2). With this technique the error of a single measurement is ±3%. The volume of venous blood sampled in each RCV measurement was approximately 5 ml. In six of the full-term and in one of the premature infants, three determinations were made and in one of the full-term and seven of the premature infants two determinations were made. In all the remaining infants one single measurement was made. The amount of ^{51}Cr injected in a single measurement was 0.05 μCi/kg body weight. This will give an absorbed dose of approximately 3–5 mrads to the blood, which presumably is the critical organ, and 0.4 mrads to the whole body (≈ the gonadal dose). The maximal absorbed dose in any of the infants of this study was less than 23 mrads to the blood and less than 2 mrads to the whole body (gonads) (cf. 2).

The venous blood was carefully mixed for 2–3 minutes in test tubes fixed to a rotating wheel before the haematocrit determination. This was performed within 10 minutes after sampling, using an International Microcapillary centrifuge, model MB. The centrifugation time was 5 minutes. The reading was made at the borderline between the red and white (or pink) cell layer. Correction was made for an assumed amount of 1.5% of trapped plasma (11).

The plasma volume (PV) and total blood volume (TBV) were calculated using the venous haematocrit and body/venous haematocrit ratio of 0.91 (2).

Table 1 *Full-term Infants*

Case no.	Age[a] (days)	Weight (g)	Hct (%)	RCV (ml)	RCV (ml/kg)	PV (ml)	PV (ml/kg)	TBV (ml)	TBV (ml/kg)
1	2	2500	67.5	138	55.2	87.0	34.8	225	90.0
	49	3480	31.5	88.0	22.7	219	56.4	307	79.1
	135	6430	33.2	137	21.3	291	45.2	428	66.6
2	3	3200	52.2	120	37.5	133	41.6	253	79.1
	46	3940	31.5	79.2	20.1	197	50.0	276	70.0
	137	5590	32.2	102	18.2	346	44.0	348	62.2
3		3520	52.2	150	42.6	166	47.2	316	89.8
	26	3530	47.1	111	31.4	148	41.9	259	73.4
	100	5690	30.1	104	18.3	276	48.5	380	66.8
4	2	3470	67.2	191	55.0	121	34.9	312	89.9
	43	4210	36.4	97.7	23.2	197	46.8	295	70.1
	129	5980	27.6	103	17.2	307	51.3	410	68.6
5	1	4240	57.6	178	42.0	162	38.2	340	80.2
	50	4600	34.0	96.1	20.9	215	46.7	311	67.6
	113	6340	31.0	115	18.1	293	46.2	408	64.4
6	3	2950	57.9	121	41.0	109	36.9	230	78.0
	61	4100	31.0	77.9	19.0	198	48.3	276	67.3
	138	5610	31.8	102	18.2	251	44.7	353	62.9
7	2	3810	46.8	155	40.7	209	54.8	364	95.5
	72	5680	28.1	108	19.0	314	55.3	422	74.3
8	0	3350	55.6	152	45.4	148	44.2	300	89.6
9	0	4500	54.2	170	37.8	175	38.9	345	76.7
10	0	4200	57.6	169	40.2	153	36.4	322	76.7
11	1	3450	53.2	155	44.9	165	47.8	320	92.8
12	1	2450	48.5	106	43.3	134	54.7	240	98.0
13	2	3630	52.7	150	41.4	162	44.8	312	86.2
14	3	2490	61.6	135	54.2	106	42.6	241	96.8
15	6	3050	55.6	134	43.9	131	43.0	265	86.9
16	7	2700	56.1	144	53.3	138	51.1	282	104
17	7	2480	51.7	98.5	39.7	110	44.4	209	84.3
18	47	3700	40.6	102	27.6	174	47.0	276	74.6
19	60	4690	29.3	92.0	19.6	252	53.7	344	73.3
20	63	4580	29.6	88.0	19.2	239	52.2	327	71.4
1	102	5600	29.6	109	19.5	296	52.8	405	72.3
22	128	6510	31.5	120	18.4	298	45.8	418	64.5

[a] Based on date of birth.

RESULTS

Clinical data of the infants and their individual values of venous haematocrit (vHct), red cell volume (RCV) plasma volume (PV) and total blood volume (TBV) are shown in Tables 1 and 2.

Venous haematocrit

The venous haematocrit values in the infants of this study were generally lower than most values reported for the same age groups (for references cf. (14)). The reason for this appears to lie in the manner of haematocrit estimation. Correction was made for trapped plasma, the white cell layer in the reading was excluded, delay was avoided between sampling and centrifugation, and careful mixing resulting in oxygenation of the blood was performed, in agreement with views expressed in a recent symposium arranged by the Committee for Standardization of Blood Cell Counts and of Packed Cell Volume Determination (1). In most previous investigations of haematocrit in infants no attention seems to have been paid to these procedures which all tend to reduce the haematocrit reading. In the full-term infants aged 60–138 days (mean age 103 days) of this study the laboratory of the paediatric clinic made haematocrit determinations of capillary blood according to their general routine, using the same centrifuge. The mean haematocrit of capillary blood (after correction for 1.5% of trapped plasma) was 33.1%. The mean haematocrit of the venous blood in these infants, as determined by the author on the same occasion, was 30.6 %.

Throughout the present work all determinations were made by the author by exactly the same technique.

Table 2. *Premature infants*

Case no.	Age[a] (days)	Weight (g)	Hct. (%)	RCV (ml)	RCV (ml/kg)	PV (ml)	PV (ml/kg)	TBV (ml)	TBV (ml/kg)
23	1	1950	52.0	75.5	38.7	81.8	41.9	157	80
	12	1870	41.4	54.2	29.0	89.6	47.9	144	[illegible]
	98	4080	34.4	74.1	18.2	163	40.0	237	[illegible]
24	1	2220	62.5	120	54.0	91.0	41.0	211	95.0
	9	2270	58.4	113	49.8	100	44.0	213	93.8
25	2	1820	49.2	76.6	42.1	94.4	51.9	171	94.0
	24	2230	33.2	61.6	27.6	142	63.7	204	91.5
26	3	1550	46.3	54.1	34.9	73.9	47.7	178	82.6
	45	2330	23.6	34.8	14.9	127	54.5	162	69.5
27	0	2300	61.8	108	47.0	84.0	36.5	192	83.5
	17	2280	50.8	83.0	36.4	97.0	42.5	180	78.9
28	1	2030	51.7	94.1	46.4	106	52.2	200	98.5
	23	2280	45.3	73.1	32.1	104	45.6	177	77.6
29	0	2340	64.0	130	55.6	93.0	39.7	223	95.3
	19	400	42.4	78.6	32.8	125	52.1	204	85.0
30	2	2080	61.3	120	57.7	95.0	45.7	215	103
	20	2350	51.7	94.0	40.0	106	45.1	200	85.1
31	0	1630	44.1	44.7	27.4	66.3	40.7	111	68.1
32	1	1920	50.7	86.4	45.0	101	52.6	187	97.4
33	1	2330	51.0	90.8	39.0	105	45.1	196	84.1
34	2	1740	62.0	102	58.6	79.0	45.4	181	104
35	2	1820	60.1	82.5	45.3	68.3	37.5	151	83.0
36	3	2100	60.1	93.9	44.7	78.1	37.2	172	81.9
37	6	2340	59.1	114	48.7	98.0	41.9	212	90.6
38	9	2190	52.2	86.5	39.5	95.5	43.6	182	83.1

[a] Based on date of birth.

Red cell volume

The changes in RCV/kg are shown in Fig. 1. The values are given in Tables 1 and 2. During the first 3 days of life the RCV of the full-term infants ranged between 37.5 and 55.2 (mean 43.6) ml/kg. In the premature infants the range was wider: 27.4–58.6 ml/kg, and the mean higher: 45.4 ml/kg. During the following weeks a similar

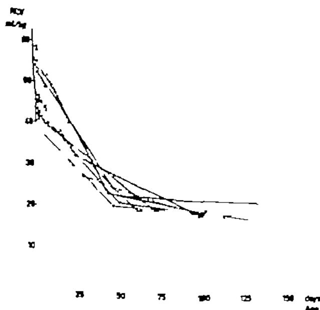


Fig. 1. Circulating red cell volume (ml/kg) in 22 full-term infants (●) aged 0–138 days and 16 premature infants (○) aged 0–98 days. Values from serial measurements in the same infant are connected by continuous line in full-term infants and broken line in premature infants.

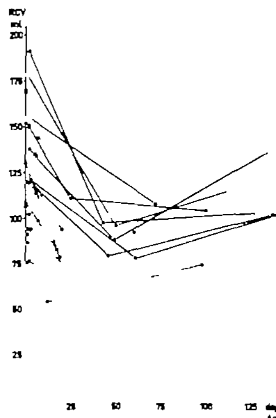


Fig. 2 Circulating red cell volume (ml) in 22 full-term infants ( ) aged *0–138* days and *16* premature infants (o) aged 0–98 days. Values from serial measurements in the same infant are connected by a continuous line in full-term infants and broken line in premature infants

Table 3 *Change in red cell volume with age*

No. of infants studied in the age interval	Age (days)	Red cell volume (% of initial RCV)
Full-term infants		
1	26	77
4	47 (43–50)	60 (53–66)
1	61	66
1	72	72
2	106 (100–113)	71 (69–74)
4	135 (129–137)	84 (57 104)
Premature infants		
2	10 (9–12)	86 (75–97)
5	21 (17–24)	77 (62–82)
1	45	65
1	98	104

change towards lower values was noted in all infants. Between the second and fourth month of age, the RCV in all infants was between 17... and 21.3 ml/kg. The mean in the full-term infants was 18 8 ml/kg. The only premature infant studied in this age interval had an RCV of 18.2 ml/kg. The corresponding values of total circulating RCV are shown in Tables 1 and and in Fig. 2.

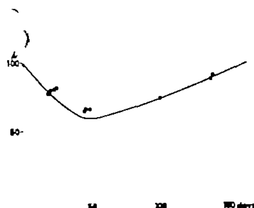


Fig 3 The change in circulating red cell volume (RCV) during the first 5 months of life. RCV measurements were performed during the first 72 hours in 8 full-term ( ) and 7 premature (o) infants. These initial values (with subtraction for the small sample volume) were taken as 100%. A second, and in some infants third, RCV (with addition of a small sample taken before the measurement) was expressed in per cent of the initial value. The *continuous line indicates the trend of change in* RCV

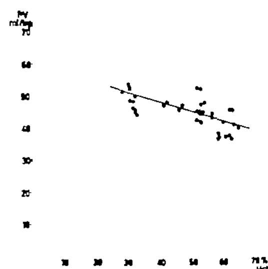


Fig. 4 The correlation between plasma volume (ml/kg) and venous haematocrit (%) in 22 full-term infants (o) aged 0–138 days and 16 premature infant (o) aged 0–98 days. The plasma volume estimations are based on measurements of circulating red cell volume venous haematocrit and body/venous haematocrit ratio of 0.91 (2). The equation of the regression line is PV(ml kg) = 60.3–0.308vHct. The standard error of regression coefficient is 0.051 The correlation coefficient is −0.63.

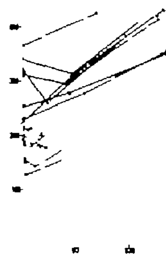


Fig. 5. Total blood volume in 22 full-term infants ( ) aged 0–138 days and 16 premature infants (o) aged 0–98 days. Values from serial measurements in the same infant are connected by continuous line in full-term and broken line in premature infants. The total blood volume estimations are based on measurements of circulating red cell volume, venous haematocrit and a body/venous haematocrit ratio of 0.91 (2).

The relationships between RCV/kg and vHct and also between RCV and gestational age have been analysed in previous studies (3, 4) where the values for the infants of the present work were included.

The results of the longitudinal st[illegible] of RCV are shown in Table 3 and Fig. 3. T[illegible]ake possible a correct estimation of the re[illegible]ge in RCV the values in Table 3 and Fig[illegible]ave been corrected for volume of sampled [illegible]ed blood cells. In Tables 1 and [illegible] and Figs. 1 and [illegible] however the measured red cell volumes are presented [illegible]th-out any correction.

In all cases studied, a decrease of RCV fr[illegible]m the initial volume, measured within the first 7[illegible] hours of life, was observed during the 6–7 weeks following birth. After these 6–7 weeks the values increased. The relative change of RCV after birth was quite similar in the full-term and premature infants.

Plasma volume

The mean calculated plasma volume of the full-term infants during the first week of life was 43 ml/kg and that of the premature infants 44 ml/kg. Between the second and fifth month the PV of the full-term infants was 49 ml/kg. A negative correlation, shown in Fig. 4, was found between PV/kg and vHct.

Total blood volume

The calculated TBV values in the individual in-fants of different ages are shown in Fig. 5. The mean value for the first three days of life was 298 ml in the full-term infants. At an age of 43–63 days the mean value was 302 ml and between 100 and 138 days this mean was 394 ml. The total blood volume per kg in the infants studied at dif-

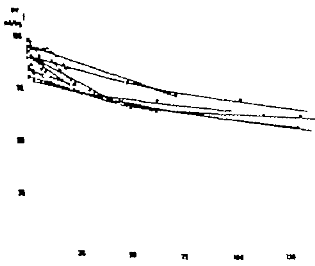


Fig. 6. Total blood volume (ml/kg) in 22 full-term ( ) and 16 premature infants ( ). Values from serial measurements in the same infant are connected by continuous line in full-term and broken line in premature infants. The total blood volume estimations are based on measurements of circulating red cell volume, venous haematocrit and body/venous haematocrit ratio of 0.91 (2).

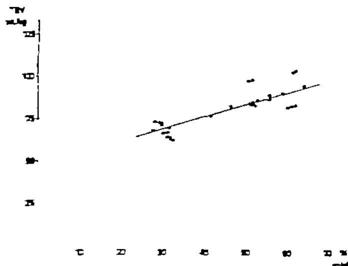


Fig. 7. The correlation between total blood volume (ml/kg) and venous haematocrit (%) in 22 full-term infants ( ) aged 0–138 days and 16 premature infants (o) aged 0–98 days. The total blood volume estimations are based on measurements of circulating red cell volume, venous haematocrit and a body/venous haematocrit ratio of 0.91 (2). The equation of the regression line is: TBV (ml/kg) = 44.. + 0.714 vHct. The standard error of regression coefficient is ±0.046. The correlation coefficient is 0.74.

ferent ages is shown in Fig. 6. During the first three days the mean of this value in the full-term infants was 86.3 ml/kg and in the premature infants 89.4 ml/kg. In the following weeks a reduction of the values was observed. The mean for 8 full-term infants aged 100–138 days was 64.. ml/kg. A positive correlation was found between TBV and vHct (Fig. 7).

DISCUSSION

..h the main purpose of the present work was to study the changes in RCV a comparison will first be made between the mean values of vHct, RCV/kg, PV kg and TBV/kg in newborn infants and infants ..-5 months old found in this study and some data taken from the literature (Table 4).

Red cell olume

A striking finding in this study is the wide range of RCV/kg (Fig. 1) at birth, with a mean value for full-term infants of 43.6 ml kg (premature infants 45.4 ml kg), in contrast to the narrow range between 60 and 138 days of age with a mean for full-term infants of 18.8 ml/kg. The wide range at birth may reflect a variation in placental transfusion. The narrow range and constant level during the age period 60–138 days seems to indicate the presence of an efficient homeostatic mechanism with respect to RCV kg.

Plasma olume and total blood volume

The PV and TBV were calculated using the body venous haematocrit ratio 0.91 as a correction factor which has been found valid in full-term newborn infants (?) and adult subjects (6). The values have been calculated merely to illustrate the alteration trend during the first 5 months of life and are not to be regarded as strict mean values for the age, since, in principle, PV should be measured by a PV method, and TBV by a PV and RCV method simultaneously.

On the average, there seems to be no great change in TBV (approximately 300 ml at birth) during the first two months (Fig. 5). An increase of about 100 ml occurs during the following two months. TBV expressed as ml kg (Fig. 6) shows a decrease from the initial level of 86–90 ml kg at birth to about 65 ml kg at the age of four months. The same pattern, with the lowest values during the first year of life at 3–4 months of age, was found in the study of Sisson *et al.* (..1). Their values, however are generally higher than those of the present work, with a mean TBV of 95 ml kg at birth and the lowest value 74 ml kg between 13 and 16 weeks of age.

The change in ed cell volume

In the work on full-term infants by Sisson *et al.* (21) the first measurement in the longitudinal study was performed within 3 days after birth in 8 infants. Four of these infants were studied again within 50 days after birth. In all of these four

Table 4. *Mean values of venous haematocrit (vHct), circulating red cell volume (RCV), plasma volume (PV) and total blood volume (TBV) from the present study compared with some data taken from the literature*

Figures printed in italics denote calculated values

Authors	Method	No. of measurements	Age (hours, days)	vHct %	RCV (ml/kg)	PV (ml/kg)	TBV (ml/kg)[a]
Full-term infants							
Present study	^{51}Cr	13	0–72 h	55.4	43.6	42 7	[illegible]
Mollison et al. (16)	^{32}P	32	0–24 h	57.0	42.2		
Mollison et al. (16)	T 1824	34	0–24 h	56.9		41.3	
Mollison et al. (16)	^{32}P T 1824	28	0–24 h	56.4			80[c]
Feldman et al. (8)	^{32}P	41	0–24 h		54 1	40 8	94 9
Sisson et al. (21)	T 1824	35	0–24 h	52.3	42.5	55.7	98
Usher et al. (24)[b]	^{125}I	9	4 h	64.1	49.5	39.5	85
Usher et al. (24)[b]	^{131}I	10	24 h	61.3	51.3	44.6	95
Usher et al. (24)	^{131}I	6	71 h	60.3	48 4	44 1	92 6
Steele (23)	^{131}I	18	2½ h	54.9	37.0	40.5	77 3
Bratteby (2)	^{51}Cr ^{131}I	4	10–48 h	55.7			88 7
Premature infants							
Present study	^{51}Cr	14	0–72 h	53.4	45 4	43.9	89 4
Schulman & Smith (19)	T 1824	(c)	(c)		46	62	108
Sisson et al. (22)	T 1824	17	3 h–6 d	50.8	43.4	59.3	103
Usher & Lind (25)[d]	^{131}I	8	0–12 h	64 8	50.5	38.3	88.8
Usher & Lind (25)[d]	^{131}I	11	12–24 h	59 1	48 8	45 8	94.6
Usher & Lind (25)[d]	^{131}I	15	24–48 h	57 1	46 7	46.7	93 3
Usher & Lind (25)[d]	^{131}I	7	48–72 h	57 4	49 4	48.8	98.2
Csorba & Jezerniczky (7)	T 1824	31	0–72 h	54	43	56	101
Full-term infants							
Present study	^{51}Cr	1..	40–138 d	30 6	18.8	49.0	67 8
Sisson et al. (21)	T 1824	16	63–77 d	30.4	20.8	62.5	83 4
Sisson et al. (21)	T 1824	9	91 112 d	31.2	19.3	55 0	74.3
Premature infants							
Schulman & Smith (19)	T 1824	9	41–44 d	26.5	13.9	57 1	71.1
Sisson et al. (22)	T 1824	14	57–82 d	28.9	22.8	72.6	95.4
Sisson et al. (22)	T 1824	14	91–111 d	28.7	19.1	61 1	80.1
Csorba & Jezerniczky (7)	T 1824	31	90 d	31	23	66	89

[a] TBV = RCV + PV

[b] Infants with delayed cord clamping.

[c] Values for the first day of life obtained by extrapolation from results of estimations made on the subsequent days.

[d] Truly premature infants.

cases the second calculated RCV was lower than the first. The mean age of these infants at the time of the second determination was 41 days, and the mean RCV was 76% of the initial value. After 50 days following birth the "residual" RCV (referred to the initial value) showed a very large variation: at an age of 62–66 days the RCV range was 43–115 % of the initial value and later the range was still wider.

In the work on premature infants by Sisson *et al.* (22) three of the infants in the longitudinal study were studied within the first three days of life. At a mean age of 39 days the mean RCV was 68% of the initial value in these infants, and in two of the infants values of 130% and 122% of the initial RCV were obtained at ages of 86 and 108 days respectively.

The findings in the present study of a decrease in RCV during the first 50 days in both full-term and premature infants, followed by a period of increasing values, are thus on the whole in agreement with the results of the two studies by Sisson *et al.* (21, 22). However the mean RCV decrease in full-term infants is somewhat larger in the present study, where 65% of the initial RCV was found in four infants with a mean age of 41 days at the time of the second determination.

Csorba & Jezerniczky (7) made serial PV meas-



Fig. 8. The change in circulating red cell volume (——) based on the present study (cf. Fig. 3) compared with the amount of initial total haemoglobin not yet catabolized ( ) at different times after birth (27) and the average survival of labelled foetal red blood cells (– – – – –) after injection into adult recipients (5). The values are expressed as per cent of circulating red cell volume and total haemoglobin mass present at birth, and injected labelled foetal red blood cells, respectively

urements on 31 premature infants within the first 3 days of life, and at 6 weeks and 3 months of age. At 6 weeks the mean of the calculated RCV for all these infants was identical to the initial RCV (74.3 ml) but at 3 months of age the mean of the differences was −8.0 ml (S.E.M. ± 3 1 ml). These results are thus in striking conflict with those obtained by Sisson *et al* (22) and the present author

In their longitudinal study on normal full term infants, Gairdner *et al* (10) made calculations of the total haemoglobin mass. These calculations were based on the haemoglobin concentration of venous blood in these infants and a prediction of TBV according to the data of Mollison *et al* (16) regarding TBV/kg body weight. The calculated daily decrease of total haemoglobin mass reported by Gairdner *et al.* (10) was 0.9%. This value, however was calculated for the age interval 9 to 29 days and is therefore not directly comparable with the results of this study where the initial value refers to the first three days. The average daily decrease of RCV in the present study was 0.9% when the second RCV measurement was performed on day 34 (26–50).

Schulman & Smith (20) calculated the daily decrease of total haemoglobin in premature infants from the first day of life to the age of 5–7 weeks to be between 1.2 and 1 4%. These values are considerably higher than the corresponding values of RCV decrease found in the present study The calculations of total haemoglobin made by Schulman & Smith were based on predicted blood volumes, according to their own regression equation of TBV/kg on age (19) and haemoglobin concentration of capillary blood. During the first weeks of life, the haemoglobin concentration of capillary blood is higher than that of venous blood. Calculations of total haemoglobin mass based on haemoglobin concentration of capillary blood will therefore lead to an overestimation of the same magnitude as the difference in haemoglobin concentration between capillary and venous blood. The discrepancy between the results of Schulman & Smith and those of the present author regarding the decrease of total haemoglobin and red cell volume, respectively would be completely explained by a difference of 28% in haemoglobin concentration between venous and capillary blood on the first day of life. Oettinger & Mills (17) found a mean difference of 21.6% on the first day whereas smaller differences have been reported by Vahlquist (26), Mollison (15) and Oh & Lind (18). Part of the discrepancy between the results of Schulman & Smith and the present author may however also be due to the fact that the former authors made estimates on more premature infants (birth weight less than 2100 g) than the present author (mean birth weight of the premature infants 2076 g).

The relation between the marked decrease in RCV during the first 50 days and two independent sets of data concerning the destruction of erythrocytes during the same period is worthy of mention. The data of Gairdner *et al* (9 10) and Garby *et al* (12, 13) indicate strongly that the production of red cells during this period is quite small. The decrease in RCV should therefore be close to but slightly less than the total destruction of cells Wranne (27) studied the haemoglobin catabolism in young infants by measuring the carbon monoxide excretion. The average calculated haemoglobin catabolism during the first 50 days of life was 51% of the initial total haemoglobin mass. Bratteby *et al* (5) studied the disappearance of foetal red blood cells labelled with DF³²P from the circulation of 8 adult recipients. A mean of 60% of the injected foetal cells had disappeared from the circulation 50 days after injection. As the calculations of haemoglobin catabolism from the carbon monoxide measurements include not only haemolysis of foetal red

blood cells but also red blood cells formed after birth, one would expect that the disappearance of labelled foetal cells should be somewhat smaller than the cumulative haemolysis calculated from the carbon monoxide data. In fact, Wranne concluded that he had probably underestimated the haemoglobin catabolism. Although he could not calculate exactly the extent of this underestimation, Wranne mentioned a correction factor which gives a cumulative haemoglobin catabolism of 58% for the first 50 days, thus very similar to the value of foetal red blood cell disappearance found by Bratteby *et al* (5) In Fig. 8 the change in circulating RCV found in the present study is compared with the results of the two studies by Wranne (27) and Bratteby *et al.* (5) referred to above.

The difference between the mean foetal red cell survival curve and the curve of RCV change during the first 2 months of life represents the volume of red blood cells produced and still not catabolized during this period. Since strict confidence limits around the two curves are lacking, the estimate of the difference between them, i.e. the red blood cell production, is uncertain. Its order of magnitude, however is compatible with the data of Gairdner *et al* (9 10) and Garby *et al* (12, 13), showing a very low rate of production.

Extrapolation of the data shows that the initial RCV is regained in full-term infants at an age of 5–6 months. One full-term infant exceeded the initial value at 135 days after birth. Only one premature infant was studied after the second month of life, and this infant had regained its initial RCV at the age of 98 days. These observations are pertinent to calculations of internal iron kinetics during this period of life.

SUMMARY

Sixty measurements of circulating red cell volume using a ⁵¹Cr method were performed in 22 full-term and 16 premature infants during the age interval 0–138 days. In 15 of these infants 2 or 3 measurements were made.

During the first 6 weeks of life the average decrease of red cell volume was 0.9% per day In this respect no difference was found between full term and premature infants.

By comparing the change in red cell volume found in this study with data from the literature on the disappearance rate of foetal red blood cells from the circulation, the red blood cell production of full-term infants during the first 2 months of life was found to be small and in accordance with previously published estimates.

The initial level of red cell volume was regained by the full-term infants at the age of 5–6 months.

The data collected also contribute to information on normal values for red cell volume, plasma volume and total blood volume during early infancy

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CONGENITAL RENAL DYSPLASIA, RETINAL DYSPLASIA AND MENTAL RETARDATION ASSOCIATED WITH HYPERPROLINURIA AND HYPER-OH-PROLINURIA

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A hereditary disturbance of amino acid metabolism, characterized by hyperprolinemia, hyperprolinuria and hyper-OH-prolinuria was described in 1962 by Schafer *et al* (12). In the proband of the family examined, the metabolic defect was associated with congenital renal dysplasia, deafness, convulsions and mild mental retardation. Three sibs of the proband had hyperprolinemia and EEG disturbances, and one of these had renal hypoplasia.

In 1965 Efron (2), described a second family with the same amino acid disturbance. Here the proband had mild mental retardation and died of uremia secondary to congenital kidney malformations. In this family however there was no nerve deafness or epileptic symptoms. A third family is mentioned by Efron (?) with hyperprolinemia but with no evidence of renal affection.

In 1964 Kopelman *et al* (5) described family with pronounced prolinuria and OH-prolinuria with only a moderate hyperprolinemia. The proband had nephrotic syndrome.

The present report describes a case which is quite similar to that described by Schafer *et al.* as it is a case with hyperprolinuria and hyper-OH prolinuria with renal dysplasia and mental retardation. The present case, however had retinal dysplasia and a mental disturbance like autism. The case is mentioned earlier in a preliminary report (9).

CASE REPORT

Lv M J was girl born at full term on Nov 30 1958. The mother suffered from nervous depression during pregnancy and took various drugs, among them chlorpromazine and paracetamol. Otherwise the pregnancy was uneventful and delivery normal. Birthweight was 3370 g. The infant appeared normal to begin with. However, during the first 6 months the mother noticed that the child did not focus her eyes and suspected that there was some visual defect. The child was therefore examined at the Ophthalmology Dept. at Rikshospitalet at the age of 7 months. On general examination at that time the consulting pediatrician found normal weight, height and general physical condition. Some rather strong deep reflexes were recorded. The ophthalmologist observed an unsteady focus as the child was unable to focus on any object. The pupils reacted to light, but possibly somewhat slowly. There was good red reflex. The fundi were not discernable. During the course of the next months the child was examined several times at the Ophthalmology Dept. A slight enophthalmos was recorded, variable undulating nystagmus and normal fundi.

During the first year of her life the child seemed happy and was content to play with her rattle. At 18 months she was able to say few words and hum bits of melody. At the same age she could sit alone, and could stand on her feet when supported.

Between the ages of 18 months and years her mental development seemed to cease, and almost to reverse. She lost her appetite, grew less communicative, lost interest in her surroundings, became more passive, and had periods of withdrawal which often ended in tears. She stopped using articulate sounds and small words. She appeared to be frightened by her own sounds, and reacted to noise in her environment by holding her hands over her ears. However, she still seemed to react positively to rhythmic sounds and could still hum fragments of simple melodies. Her motor development seemed almost arrested at this time. She never learned to walk alone, and she stood alone only on special occasions when she was very happy such as when she could play with water.

At the age of 5 years she was admitted to home for mentally retarded. Here she was kept under careful observation, particularly to determine whether she was blind

Table 1 *The urinary excretion of free amino acids and other ninhydrin positive substances/g creatinine in the actual case*

	mg/g creatinine	Normal mean	Normal range
Lysine	23		0–25
Cystine	9	19	0–50
Glycine	69	58	10–150
Histidine	69	148	50–250
Alanine	35		0–70
Glutamine	58	49	0–100
Proline	115	0	
OH-proline	46	0	
3-methylhistidine	28	42	0–150
Tyrosine	14	13	0–50
Tryptophan	7		0–5
Phenylalanine	12		0–10
Taurine	9	77	0–250

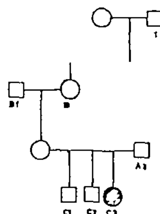


Fig 1 The pedigree of the family with the hyperprolinuric child.

Most of the people who cared for her thought that she could distinguish light from dark. Her parents also believed that they had occasionally had very brief visual contact with her and this seemed to bother her. She would rub her eyes vigorously let her eyes wander and turn away It is really remarkable that during the six years of her life it had never been possible to decide conclusively whether she had any sight, or whether her lack of focus was an autistic symptom. At the time her condition was interpreted as "autism, possibly due to brain injury in addition to amblyopia of unknown origin

Even before admission to the mental institution there had been feeding problems. She ate only dry things like crackers, and fruit. She had an [illegible] for any kind of cooked, damp food. She developed anemia and on June 20 1964 she was admitted to the Pediatric Dept at Rikshospitalet for closer observation and especially for biochemical tests.

Laboratory findings. Blood. ESR 80 mm/h, Hb 58%–48% erythrocytes 2.88–2.53 mill/mm³ leucocytes 12.000/mm³ with normal distribution, thrombocytes 188.000/mm³ serum protein 7 7 g/100 ml, serum iron 34 μg/100 ml, serum cholesterol 272 mg/100 ml, serum lipids 1250 mg/100 ml. Electrophoresis. albumin 3.8, alfa₁-globulin 0 4 alfa₂-globulin 1.8 beta-globulin 1.0 gamma-globulin 1.5 g/100 ml. Urine. trace of albumin, sp.wt. 1.014 Benzidine reaction negative, Clinistix negative, microscopy normal, no bacterial growth from urine Pulmonary X-ray normal. EEG cerebral activity below normal for age?

Her refusal of food continued, and in spite of parenteral iron administration her Hb sank to 45%. She had periods of diarrhea, developed a peculiar pale yellow complexion, and readily developed suggillations when bruised slightly

She was again admitted to the Pediatric Dept. on Dec. 15 1964. At that time her muscles were somewhat atrophic, but quite strong. Ophthalmologically spontaneous nystagmus was found with horizontal rotatory phase to the right. Pulse was 100, blood pressure was not measured. Deep reflexes were rather strong. Otherwise the general examination revealed normal findings.

Laboratory findings. Blood. ESR 70 mm/h, Hb 55%, erythrocytes 3.16 mill/mm³ leucocytes 12,900/mm³ reticulocytes 1.5% thrombocytes 263,500/mm³ osmotic resistance normal. Bone marrow sparse erythropoiesis. Serum iron 113 μg/100 ml, T. I. B. C. 268 μg/100 ml, serum Na 137 mEq/l, serum K 4.6, mEq/l, serum Cl 105 mEq/l, serum Ca 4.8 mEq/l, serum P 6.4 mg/100 ml, serum urea 200 mg/100 ml, serum creatinine 3.7 mg/100 ml, alkali reserve 13 mEq/l, pH in blood 7.18, P_{CO_2} 35 mm Hg. Urine: trace of albumin, Clinistix positive, microscopy showed hyaline and granular casts, 10–20 white cells per field of vision.

During this period her condition was interpreted as a chronic pyelonephritis with secondary anemia and she was treated with sulfonamide

Her condition gradually deteriorated. On Jan 20, 1965 the excretion of amino acids in her urine was examined, and it was found that she excreted large quantities of proline and OH-proline. She died on May 14, 1965, at the age of 6 years and 5 months, before any detailed studies of her amino acid metabolism could be carried out.

Table 1 shows the results of [illegible] this layer chromatographic determination of her excretion of amino acids and other ninhydrin positive substances. The method of semiquantitation has been published elsewhere (8) An investigation of normal values at different age groups is to be published (10).

The most striking feature of the table is the excessive excretion of proline and OH-proline Furthermore, the excretion of lysine, tryptophan and phenylalanine lies in the upper range. The sugar chromatogram of the same sample of urine was normal

FAMILY HISTORY

The pedigree of the family is shown in Fig. 1

A 1 Paternal grandfather some "nervous troubles

A 2 Paternal grandmother rheumatic joints, periodic mental disturbances, difficult, bad tempered, anxious She had 1 children, three of whom died in early childhood

A 3. Father born Febr 5 1923. Suffered from headaches during youth Some education beyond primary

what Sandy and a hard worker. Employed at the same place for the last 17 years in a warehouse. Urine: no excessive excretion of proline or OH-proline. Microscopy normal.

B 1. Maternal grandfather alcoholic. Several confinements in psychiatric hospital, with 21 shock treatments. Died at 60 of coronary infarction.

B 2. Maternal grandmother on the whole healthy but often worn out and nervous.

B 3. Mother born March 31 1926. Daughter / her husband' eldest sister in other words the daughter of one of the daughters of A 1 and A 2. Nervous breakdown at 22. Duodenal ulcer at 28 and 29. Depression during last pregnancy aged 33. Since then constant attacks of anxiety and several admissions to psychiatric hospital. Urine: no excessive excretion of proline or OH-proline. Microscopy normal.

C. Children of A3 and B3

C 1. Ivar born Oct. 10, 1946. A quiet, sensitive boy frequently troubled with headaches. Encopresis until age of 5. Enuresis up to 15 years of age. Some dyspepsia. X-ray: deformed bulbus duodeni, duodenum superior looped. EEG normal. Eyes and ears: normal. X-ray heart and lungs normal. Renal examination with urography creatinine clearance, serum creatinine: normal. Urine: No excessive excretion of proline or OH-proline. Microscopy normal.

C 2. Ruben, born April 28, 1951. A bright, well-behaved, pleasant and sociable boy. Congenital heart defect which was judged after catheterization to be an atrial septum defect combined with an Ebstein anomaly i.e. a low attachment of the tricuspidal valves, which are insufficient.

Ophthalmological examination revealed considerably reduced vision of the left eye, but normal fundi. Ears were normal. EEG showed slight indication of diffuse, extensive slow activity of 4-6 sec frequency. Renal examination with urography creatinine clearance and serum creatinine: normal. His urine revealed the inconstant phenomenon of somewhat greater number than usual of cells which were interpreted as tubule cells, but otherwise his urine was normal. No excessive excretion of proline or OH-proline.

C 3. Liv M born Nov 30, 1959: Index case

AUTOPSY

Bronchopneumonia was found bilaterally.

Kidneys. The kidneys weighed each approximately 40 g. The surface was uneven with U-shaped depressions. The cut surface of the left kidney showed reduced and indistinctly outlined cortical tissue and the colour was brownish-red. The right kidney had lighter brownish-... cut surface, irregular without marked borderlines between cortex and pyramids. The papillae were hardly noticeable.

Microscopical examination showed similar picture in both kidneys, the changes being only more pronounced on the right side. There were many well preserved glomeruli in addition to others that were rudimentary and surrounded by dilated capsular space. The Bowman capsule was mostly thickened. The endothelial lining was smooth without any signs of crescent ... Small cysts filled with pale staining fluid were lined by the same endothelium. A few groups of convoluted tubules, slightly dilated, were seen, otherwise the tubules appeared atrophic, lined by cuboidal epithelial cells and surrounded by markedly increased connective tissue fibers often arranged concentrically. A number of ... tracts were also lined by cuboidal epithelial cells. In ... ally increased interstitial tissue, lymphocytes ... in groups perivascularly and also to some extent scattered in the tissue. The picture corresponded to that of Renal dysplasia with chronic pyelonephritic changes.

Central nervous system. Grossly the brain was seen covered by thin and translucent meninges. The gyri appeared somewhat uneven and in both parietal regions slightly depressed. The right temporal lobe seemed to be slightly smaller than the left. Sections from the cerebral cortex and corpus striatum showed on microscopy edema, poorly differentiated cells with scanty cytoplasm. Large pyramidal cells were not seen in the area striata. The thalamic nuclei were quite well preserved, and the neurons were here of usual size and well stained. In the lateral geniculate body the lamination was indistinct, and the cells seemed atrophic.

Sections through various levels of the spinal cord showed the fasciculi to be generally reduced. In the lumbar region the anterior horn motor cells appeared normal, while they in sections from the cervical and thoracic levels were few and atrophic. A few small perivascular blood extravasations of the agonal type were present in the grey substance.

Sections through both eyes displayed mainly the same picture. The sclera was thick and fibrous. In the choroid scattered pigment laden fibroblasts were seen, while the pigment layer contained less pigment than usual. The retina was not well preserved, but there was marked atrophy. The outer granular layer was mostly reduced, in some places to single row of ependymal like cells. The outer limiting membrane could not be discerned. There was scattering of pigment laden glial cells between the inner granular cells. In the ganglion cell layer some ganglion cells were seen, and the fiber layer was partly preserved. The optic nerves were atrophic bilaterally with increased connective tissue trabeculae.

Diagnosis: Brain and spinal cord with inhibited development. Retinal dysplasia.

COMMENTS

The urine specimen for the amino acid investigation was taken on Jan. 20, 1965 one month after the patient's stay at Rikshospitalet and about 4 months before she died. At this time she had progressive renal failure with uremia. Efron (2) claims to have seen patients with severe renal disease and prolinuria and OH-prolinuria. We have, however examined urine from patients with advanced renal failure and serum creatinine up to

Table 2. *Comparison of findings in two prolinuric patients, their parents and sibs*

Schafer *et al.* s case (12)	Our case
Increased excretion of proline, OH-proline and glycine	Increased excretion of proline, OH-proline and possible lysine, tryptophan and phenylalanine
Increased plasma proline (7 9 mg/100 ml as compared to about 3 mg/100 ml in parents)	Not determined
Increased proline and OH-proline excretion in 2 of the patient s 5 sibs	No increased proline or OH-proline excretion in parents or 2 brothers
Microscopic hematuria in patient, 2 sisters nd m mother	Inconstant increase of tubular cells (?) in urine of one brother
Monolateral renal hypoplasia in patient	Bilateral renal hypoplasia in patient
Urographically demonstrated unilateral renal hypoplasia in mother and one sister of patient	Urographically normal findings in patient s 2 brothers
Pathological EEG in patient as well as mother and 3 sisters	Pathological EEG in patient and one brother
Defecth hearing i patient, mother and one sister	N observation or information of defective hearing in patient, her parents or sibs
N record of impaired vision	Retinal dysplasia with severely reduced sion(?) in patient. Reduced vision in one eye otherwise normal ophthalmological findings in one brother
atient mentally retarded	Patient mentally etarded
Patient developed pyelonephritis	Patient developed chronic pyelonephritis
Patient died ged 5 years and 8 months	Patient died aged 6 years and 5 months

28 mg/100 ml, but none of these showed any similar excretion. It would, indeed, be improbable that her pyelonephritis would affect the specific mechanism for the reabsorption of the prolines, while the excretion of the other amino acids remain largely within normal ranges. The metabolic defect as regards amino acids is therefore assumed to be a primary affection.

Table 2 shows a comparison between the findings in Schafer *et al* s patient and our own case. There is a striking similarity between the findings and the course of the disease in these two patients. Both are mentally retarded with a congenital renal dysplasia and with proline- and OH-prolinuria. Both died at about the same age of pyelonephritis As our case died before we had an opportunity to do further investigations, we cannot say whether she had a hyperprolinemia. The similarity is nevertheless so great that there is a strong possibility that both patients suffered from the same disease as regards the amino acid metabolism. The most prominent differences between them are the impaired hearing in one case, while the other had retinal dysplasia and possibly impaired vision. Further it is the gravity and the type of the mental disturbances. This was very mild in the first case, very grave in our case and with a peculiar picture of *autism*

The study of Schafer *et al* of the distribution of the various symptoms in the patient s family led to the conclusion that it is possible that the deafness, nephropathy and EEG disturbances are inherited from the mother while the amino acid defect is the result of an interaction with a factor from the paternal side. If the defect in amino acid metabolism in *our* case is due to the same mutated gene as in the other case, it would appear that the amino acid metabolic defect and the nephropathy have the closest genetic association, while the deafness and the retinal dysplasia may be determined by contributing factors.

There is consanguinity in our case, as the patient s father is his wife s uncle. This supports the assumption that the disease is caused by a recessive gene.

Schafer's report makes no mention of any psychiatric disturbance in the patient's family In our case there is quite an accumulation of nervous diseases in the family This should be noted, as a heterozygote may have some hitherto undemonstrated biochemical defects which may condition the individual for psychiatric diseases.

As mentioned above, the diagnosis for our patient until the last year of her life was *autism*. It is of interest to note that a congenital amino acid defect may cause a developmental picture with the form of autism.

Hereditary progressive renal disease has been described previously (7) It has also been reported in connection with anomalies in other organs (1 11). Several authors have observed that the pyelonephritis can develop on the basis of renal dysplasia (3 4 6) The association between renal dysplasia and blindness was first described by Christie Løken *et al* in 1961 (1). This was a pair of sibs who died at the ages of 8 and 9 years of renal failure. One of these was mentally

Fig 2 Degra[illegible] pathway of l-proline me[illegible]

retarded. The findings and the course of the disease in these two cases is so similar to our own that it might have been identical, but no investigation of amino acid excretion was made in their cases.

Fig. 2 shows the degradative pathway of l-proline. In Efron's case, type I, which is similar to our case, the biochemical mechanism for the prolinemia and prolinuria was shown to be a deficiency of proline oxidase (2). In the case with no evidence of renal disease, type II, the defect was assumed to be a deficiency of Δ pyrroline 5-carboxylic acid dehydrogenase. The coexisting OH-prolinuria may be due to a saturation of a common tubular transport mechanism by proline. We got no opportunity to investigate our case as to the same mechanisms.

It is not known what connection there may be between the various abnormalities observed, renal dysplasia, retinal dysplasia, defective proline metabolism and disturbances in the development of the central nervous system. In our case it seems that the cerebral involvement became aggravated from about 18 months of age, while the visual impairment was probably present earlier. This may mean that renal dysplasia, retinal dysplasia and the metabolic proline defect are primary while the cerebral involvement progresses under the influence of the defective amino acid metabolism. It would therefore appear to be important that children with possibly defective vision during the first year of life, as well as children who seem to suffer from autism, be examined for amino acid excretion, so that this defect can be detected before it causes permanent brain damage.

As for therapy dietary treatment is less promising because the prolines are non essential amino acids. Intermittent hemodialysis might be a possibility.

SUMMARY

A case is described with prolinuria and OH prolinuria associated with congenital renal dysplasia, retinal dysplasia and mental retardation. The mental disturbance showed a peculiar picture of autism. Consanguinity supports the [illegible]u[illegible]tion that the disease is caused by a reces[illegible] gene. There was an accumulation of nervous dise[illegible]es in the family. This raises the question whether one recessive gene may condition the individual f[illegible]r psychiatric diseases.

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REVIEW ARTICLE

POLYNEUROPATHY IN CHILDHOOD

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Impaired function of peripheral nerves may be difficult to demonstrate on neurological examination of young children. Measurement of the conduction velocity of peripheral nerves offers an objective method for demonstrating impaired function also in patients who are unable to co-operate. This method may disclose evidence of peripheral neuropathy in patients in whom this disorder otherwise may be masked by symptoms due to simultaneous involvement of the central nervous system. Clinical neurological examination supplemented by this method revealed 43 cases of polyneuropathy in children below 17. The clinical picture and the results of special studies are described and discussed.

MATERIAL AND METHODS

The present material was collected during the years 1961–67. Most of the cases were seen at the Department of Pediatrics, Lund and a few at the Children's Memorial Hospital, Chicago.

The conduction velocity of the motor nerve fibres of the ulnar, median and peroneal nerves was measured on both sides, the peripheral sensory conduction time of both median nerves was also measured in many of the patients. Details of the methods used and the values considered normal, borderline and abnormal in different age-groups have been reported previously (10, 11, 13, 14, 36). In many of the patients conduction velocity was abnormally low in all the nerves examined. At least 2 abnormal values or 4 borderline values plus minor clinical findings were considered evidence of polyneuropathy. Two of the patients, 2 diabetic girls with absent ankle jerks and unequivocally impaired vibration sense on both feet did not fulfil these criteria. The clinical evidence of polyneuropathy was considered strong enough to include them in the material in spite of their normal conduction velocity. Polyneuropathy is thus not used as a diagnosis. Under this heading are included various cases with the common finding of impaired function of at least peripheral nerves.

The material consisted of 43 children, 27 boys and 16 girls, below 17; the youngest was a newborn. Beside the studies already mentioned several genetical, clinical, laboratory, neurophysiological and histopathological examinations were carried out in an attempt to establish, whenever possible, a definite diagnosis, the cause of the neuropathy and the extent of the involvement of the nervous system.

RESULTS

The material was divided into _ main groups: children *with* signs of co-existing involvement of the central nervous system and children *without* such signs (Table 1).

In one instance it could not be decided to which group the patient belonged. The patient was a newborn boy with severe muscular hypotonia and areflexia. Conduction velocity measured for the first time at age 5 days, was between 1 and 5 m/sec (normal for age about 30 m/sec). Neither the cause of polyneuropathy nor the further course in this boy is known; his mother reported that she had taken various drugs during pregnancy; their names were not known to her.

Table 1. *Children with polyneuropathy*

	Boys	Girls	Total
With signs of involvement of CNS	10	3	13
No signs of involvement of CNS	16	13	29
Uncertain (newborn)	1		1
Total	27	16	43

Involvement of the central nervous system

Table 2 surveys the children with definite evidence of involvement also of the central nervous system. One or more of the following findings were made in these children: convulsions, pyramidal tract signs, cerebellar ataxia, bulbar palsy mental changes, unequivocal EEG-abno malities. Two had *late-infantile metachromatic leucodystrophy*: the clinical and laboratory findings were typical and the diagnosis was proven by histological examination of nerve biopsy specimens. One boy had *rubella* *severe neurological complications*. About week after the appearance of the rubella rash he developed paraplegia and bladder paralysis. About eek later he had paresthesia also in his hands and severe EEG-abnormalities. When he was examined about 3 week after the onset of neurological symptoms, the conduction velocity was

Table 2. *Children with polyneuropathy volvement of CNS*

	Boys	(	Total
Late-infantile metachromatic leucodystrophy	1	1	
Rubella encephalo-myeloneuropathy	1		1
Friedreich ataxia	2		
Metabolic	1		1
Unknown cause			
Ataxia and convulsions	1		1
Encephalomyeloneuropathy			
Progressive	2	1	3
Non-progressive	1	1	2
Encephalomyeloneuromyopathy	1		1
Total	10	3	13

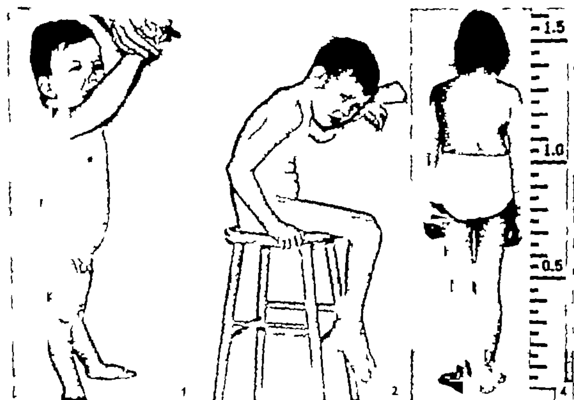


Fig. 1. Boy aged 2, with muscular weakness and hypotonia, due to polyneuropathy and signs of involvement of the central nervous system.

Fig. 2. Boy aged 7 h muscular weakness and hypotonia, due to polyneuropathy and signs of involvement of the central nervous system, such as drooling caused by swallowing difficulties due to bulbar palsy.

Fig. 4. Kyfoscoliosis in girl of 14 with chronic hereditary polyneuropathy.

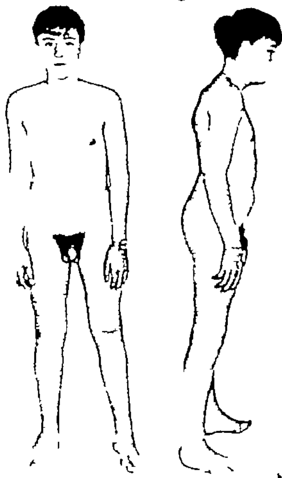


Fig 3 a and b. Excavated feet in boy of 14 with chronic hereditary polyneuropathy Wasting of the muscles of lower legs is slight. No wasting of the hand muscles.

Table 3 *Children with polyneuropathy*

No signs of involvement of CNS

	Boys	Girls	Total
Diabetes mellitus	5	6	11
Classical acute Guillain–Barré	2		2
Positive family history	9	4	13
Unknown		3	3
Total	16	13	29

mildly decreased in the arms and severely abnormal in the legs Later the peroneal nerves became inexcitable and electromyography showed total denervation of his leg muscles. The symptoms and signs, both clinical and neurophysiological, in his arms disappeared. A pair of identical twins with *Friedreich ataxia* had areflexia and muscular atrophy of the lower legs; conduction velocity was low in the peroneal nerves on both sides and normal in the nerves of the arms. The metabolic case occurred in boy with *tyrosinosis*. This case has been reported by Aronsson *et al.* (1) When this diagnosis was established the boy was 1 year old and clinical neurological examination and measurement of conduction velocity at that time had revealed no abnormalities. He was given tyrosine-poor diet, extra vitamin D and an oral multivitamin preparation. He always ate poorly. At age 5 he developed signs of liver cirrhosis, had grand mal seizure and severely abnormal EEG He became dyspnoic and very weak. *His muscle reflexes disappeared and his muscles became atrophic.* Electromyography revealed wide-spread severe denervation. Conduction velocity was low in 2 nerves, the other nerves were inexcitable. He was treated with intramuscular injections of vitamin B and within a couple of weeks he began to improve. After 6 weeks therapy clinical improvement was considerable, but the conduction velocity remained unchanged.

One boy of 14 had, beside polyneuropathy convulsions, *EEG-abnormalities and cerebellar ataxia.* He was followed up for 4 years with no obvious progress of symptoms or signs. The 2 patients with non-progressive encephalomyeloneuropathy had *an acute reversible disease.* In the boy the clinical picture was that of a Guillain-Barré syndrome and involvement of the central nervous system was detected, because an electroencephalogram, routinely performed in all children with this clinical syndrome, revealed unequivocal abnormalities. The girl had severe brain stem symptoms, she was unconscious and in a respirator for 3 weeks. Thus, in her case initial severe symptoms from the central nervous system were followed by evidence of involvement also of the peripheral nerves.

The group progressive encephalomyelopathy included one girl, who at age 3 months started to develop clinical symptoms and laboratory findings typical of *Krabbe's disease.* However her muscular hypertonia was replaced by hypotonia, the previously hyperactive muscle reflexes disappeared and her conduction velocity was found to be low Histological examination of nerve biopsy specimen revealed unspecific demyelinisation, but no metachromatic substance. The 2 boys in the same group were brothers who had from infancy shown slow motor development, muscular hypotonia and weak reflexes. Pyramidal tract signs, cerebellar ataxia, bulbar palsy and progressive mental changes supervened during the following 5–6 years. No specific diagnosis could be established, *chronic necrotizing encephalopathy localized to the brain stem (chronic Wernicke-encephalopathy)* was considered a possible explanation. Histological examination of muscle biopsy nerve biopsy and rectal biopsy in one of them revealed no specific abnormalities. Fig 1 shows the younger boy at age 2, and Fig. 2 the older boy at age 7.

In one boy muscular weakness and wasting of thigh muscles, electromyographically proven to be due to *primary muscle disease* was found beside *low conduction velocity slow clumsy movements, cerebellar ataxia and mental retardation.* No explanation for this complicated neurological disease was found, the ataxia and the neuropathy improved shortly after therapeutic trial with large doses of the vitamin B-complex.

No apparent involvement of the central nervous system

In 29 children no evidence could be found of co-existing involvement of the central nervous system (Table 3). Eleven had *diabetes mellitus*. These children came from an unselected group of 107 diabetic children deliberately examined for possible signs of polyneuropathy: thus none of them had subjective complaints. Details about these patients have been reported (11, 14).

Two children had a classical *Guillain-Barré syndrome* without evidence of central involvement.

In 13 children belonging to 9 different families a *positive family history* was obtained. In 6 of these families one of the parents was known to have the same disease, 5 of them could be examined and the diagnosis was confirmed in all of them. In a 7th family the history was said to be negative, but examination of both the parents revealed evidence of the disease in the mother. In 2 families, each with a pair of affected sibs, both clinical and neurophysiological examination of both parents in one of the 2 families revealed no abnormalities, the other parents were described as healthy and were unavailable for examination. In this group of hereditary polyneuropathy the disease ran a slow chronic course with a variable clinical picture. Three sibs had no symptoms or clinical signs. Their father had chronic polyneuropathy and belonged to a family with many affected members. His 5 children were therefore examined and an abnormal conduction velocity was found in 3 of them. They were followed for 3 years and re-examined twice with the same result; at the latest examination the oldest boy (age 19) had started to have symptoms and positive clinical signs. In most of the children the first symptoms, noticed at age 2–4, were a stumbling gait, frequent falls, difficulties to run, and slow clumsy movements. These together with excavated feet and hypoactive or absent muscle reflexes were also the findings made on clinical examination (Fig. 3). From age 6–8 the children turned the feet more and more inwards; some atrophy of small foot muscles was usually also noted. Atrophy of the small hand muscles was not observed before age 12–14, when a few of the children also showed wasting of lower leg muscles. Unequivocal loss of sensation could never be demonstrated, although a few of the older children probably had some mild disturbances. Kyphoscoliosis was found in 4 of the children aged 1.–16 (Fig. 4). Among these 4 was also the most severely affected patient in the group, a girl, who had weakness and wasting not only of the distal but also of the proximal limb muscles, although the distal muscles were more severely affected than the proximal ones. She could thus not climb stairs, not get up from a chair without using her hands and barely walk without support. She improved slightly after treatment with anabolic steroids. Hereditary syndromes with polyneuropathy as a part of the clinical picture such as porphyrinuria, Refsum's disease, were as far as possible excluded.

In 3 patients, all girls, the family history was negative and examination, including measurement of conduction velocity of parents and sibs revealed no abnormalities. In one of them the course was similar to the usual type in the hereditary cases with the first symptoms noticed around age 2 and slow clumsy movements and areflexia as the dominating findings. Another girl was healthy to age 9 when, after a throat infection, she developed muscular weakness, first and always most marked in distal limb muscles. She deteriorated, and also proximal muscles became weak. Her disease ran a fluctuating course for about 4 years, after which she began steadily to improve. Steroids and cytostatics were tried with no apparent effect. The third girl's symptoms started at age 8. Her subjective symptoms and the clinical findings were always confined to the right thumb and index finger. A mononeuropathy caused by pressure on the median nerve in the forearm was first diagnosed and operated on, but later measurement of conduction velocity of other nerves revealed polyneuropathy. The conduction velocity of all nerves except the right median became normal 3 years after onset of symptoms, by which the symptoms and signs in the right hand were improving.

In 4 patients the enzyme activity (transaminases, lactic dehydrogenase with isoenzyme studies and creatine kinase) in the serum was measured and found to be normal in all. The spinal fluid protein, examined in 7 patients, was elevated in 4. Electromyography of distal muscles performed on 10 patients revealed some signs of denervation in all. In 6 children a biopsy specimen was taken from the anterior tibial muscle; histological examination revealed no abnormalities in 3 patients and evidence of denervation in 3 (Fig. 5); in one of them findings usually interpreted as signs of a primary myopathy were also noted. A biopsy specimen of the sural nerve was taken from 3 patients; histological examination disclosed unspecific, degenerative changes, in one patient with fibrosis (Fig. 6).

Unless otherwise stated physiotherapy and orthopedic measures were the only treatment given.

DISCUSSION

The occurrence of polyneuropathy in young infants has been reported previously and stressed as a possible cause of muscular hypotonia in infancy (3, 4, 19). A correct diagnosis in this age-group is important mainly because polyneuropathy appears to have a better prognosis than several of the other conditions which may cause muscular hypotonia in infancy (3, 4, 29).

When the central as well as the peripheral nervous system is involved, the resulting clinical picture may be difficult to interpret. The muscular hypotonia and diminished reflexes in peripheral neuropathy may be masked by spasticity and a tendency to increased muscle reflexes caused by a pyramidal tract lesion, and a cerebellar ataxia may obscure a sensory ataxia. Children with symptoms and signs suggesting a disease of the white matter of the central nervous system were

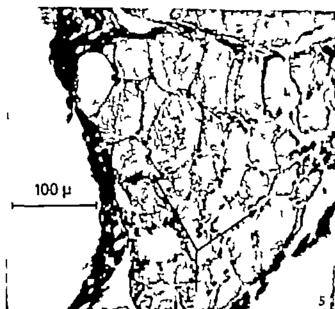


Fig 5 Biopsy specimen f the anterior tibial muscle in the girl shown in Fig 4 The arrows indicate groups of angulated, atrophic fibres, characteristic of denervation. (× 225 Hansen–v Gieson)



Fig 6. Biopsy specimen of the sural nerve in a girl of 16 with chronic hereditary polyneuropathy Some axons are blown up or fragmented (arrows) with concomitant rarefaction of the myelin sheaths. Little or no cellular reaction. (160, Bodian stain for axones).

therefore systematically examined for evidence of involvement also of the peripheral nerves. The involvement of the peripheral nerves in *late-infantile metachromatic leucodystrophy* is well known (9 16 18) and measurement of conduc ion velocity has been suggested as a screening procedure (5). The disease may however also start as a polyneuropathy with low conduction velocity and barely any signs of involvement of the central nervous system (38) histological examination of a nerve biopsy is necessary to establish the diagnosis.

To my knowledge involvement of peripheral nerves has not been reported in *Krabbe's disease* although patients have been described in whom muscular hypotonia has been observed at some stage of the disease (15). In the present case the diagnosis, though not proven by autopsy appeared well established from the repeated typical finding of a high total protein and a relative increase of the albumin content of the spinal fluid. Histological examination of a nerve biopsy specimen revealed unspecific demyelination but no metachromatic deposits, thus excluding a diagnosis of metachromatic leucodystrophy In this connection it should be mentioned that conduc tion velocity was measured and found to be normal in 6 other children with clinical signs of a white matter disease but with normal spinal fluid protein and no evidence of leucodystrophy

In *Friedreich's ataxia* peripheral weakness and wasting are often seen together with hypoactive or absent muscle reflexes. Involvement of the anterior horn cells, anterior roots or as in the 2 patients presented here, of the peripheral nerves, has been reported. The localization varies from patient to patient and at different levels in one and the same patient (33) One more patient with Friedreich's ataxia was seen during the same period she had a normal conduction velocity despite peripheral weakness and areflexia. The relation between Friedreich's ataxia and polyneuropathy has been extensively discussed in the literature (17 3., 33).

Poor absorption or an increased demand of vitamin B causing a *B-hypovitaminosis* seem the likely explanation of the neurological symptoms and signs in the boy with *tyrosinosis* this prompt response to parenteral administration of vitamin B lends support to such an interpretation.

Cases of an acute disease with involvement of both the central and the peripheral nervous system, occurring *for unknown reason or after a viral infection*, have been reported previously

Two of the patients briefly mentioned here have been described elsewhere (2), where the problem was discussed in detail.

In the 2 brothers with a progressive neurological disorder a *necrotizing encephalomyelopathy* (21) was suspected. Symptoms from the lower brain stem were predominant, particularly in the older brother but both brothers had been weak and hypotonic from an early age. Muscular hypotonia has been reported as a prominent feature (20), and hypoactive or absent muscle reflexes have been described (8, 21). In 2 patients on record clinical and electromyographical evidence of a lower motor neurone disease was strong (31). Conduction velocity was measured in one of them and said to be normal, but the figure given, 28 m/sec, is abnormal for the age of the child, provided a standard method was used. Histological examination of peripheral nerves revealed no abnormalities in one of the patients and only minute foci of demyelinization in the other. The findings in these 2 patients were thus similar to those in the brothers presented here. Reye (30), however found widespread demyelination of peripheral nerves in 3 of 4 cases studied with neuropathological examination also of the peripheral nerves.

In the boy with both *a primary muscle disease and an ffection of the central and peripheral nervous system* the underlying mechanism remained unknown in spite of extensive examinations.

Impaired nerve function was demonstrated by the methods used here in about 10% of *juvenile diabetics*. The same incidence was found in a similar study by Eeg-Olofsson & Petersén (7). The incidence is higher in adults (25) also when the material is limited to patients with a short duration of the disease.

The present material included only 2 patients with a classical *Guillain-Barré syndrome*. Several reviews on the condition in childhood are on record (23, 4 28, 34 37).

The largest group, 13 patients, belonging to 9 families, consisted of children with cases of *chronic polyneuropathy in the family* previously known or found on systematic examination of the family. The findings indicated dominant inheritance in 7 families and suggested recessive inheritance in 2. Besides, the course of the disease in one of the girls in the unknown group was identical with that seen in most of the hereditary cases. Although neither her parents nor be   had symptoms, clinical findings or abno   duc tion velocity it is possible that   had hereditary polyneuropathy with re   eritance.

Impaired hearing, nystagmus, pupi maldies, cerebellar ataxia, and enlar were not found in the children with ch reditary polyneuropathy The clinical varied in this group. In 3 asymptomatic ch aged 7 16, with no clinical signs, the disea detected through systematic measurement o th conduction velocity in the 5 children of a patien known to have hereditary polyneuropathy. Dyck *et al* (6) and Myrianthopoulos *et al* (26) h studied large kinships with Charcot Mari Tooth's disease, stressed the diagnostic value of low conduction velocity in the preclinical stage of the disease.

In one boy the disease was found at a routine school health examination, as he had excavated feet, an awkward gait and areflexia. Examination of the family revealed the disease in the mother in an adult sister and in a small brother. Even the adult members of the family appeared unaware of any disease in the family

The history given was similar in the remaining 9 cases. The parents became aware of something wrong, when the children at age 2 3 continued to walk clumsily and to fall often and did not learn to run. It should be stressed that delayed motor development, slow clumsy movements, hypoactive or absent muscle reflexes, excavated feet and, in some patients, kyfoscoliosis were the typical findings in the children, although their affected parents had the clinical picture characteristic of Charcot Marie Tooth's disease. This was also seen in one of the children examined for the first time at age 14. The clinical picture of the younger children thus deviated from that usually seen in chronic polyneuropathy in adolescence and adulthood. The same clinical picture has been described previously (22) and particularly stressed as characteristic of chronic polyneuropathy in childhood (3). One girl, examined for the first time at age 15 was the most severely affected in the present material. She had experienced a severe progress of symptoms at puberty when also her proximal limb muscles and trunk muscles became weak; the distal muscles were, however always more severely affected than the proximal ones.

She was referred to us under the diagnosis of muscular dystrophy Byers & Taft (3) reported the same development and the same misdiagnosis in some of their patients.

The paucity of sensory symptoms is not surprising in a disease with such an early and insidious onset; a mild sensory loss is difficult to demonstrate in a child, in whom the sensation has perhaps never been normal. The motor ineffectiveness with slow clumsy movements is, however, out of proportion to the muscle weakness and is interpreted as evidence of a sensory element in the patient's disability (3). The loss of muscle reflexes already at a stage when muscle weakness is mild, points in the same direction, as it might be due to affection of both the sensory and the motor paths in the mixed peripheral nerve.

In one of the remaining 2 patients in whom *no explanation* could be offered for the polyneuropathy the onset of the disease was rather acute following a sore throat, and the course was protracted and fluctuating. This picture has been described previously in children (27 28) Steroids and cytostatics were tried with no apparent effect.

In the other patient with polyneuropathy of unknown cause the clinical symptoms and signs were confined to the right median nerve. In this child, as in the adults reported by Thage *et al* ( ) neurophysiological examination revealed an extensive involvement of peripheral nerves in a patient, who clinically appeared to have an affection of one nerve only

Laboratory examinations were of little diagnostic help in the patients without evidence of involvement of the central nervous system. Normal serum enzyme activity found in the few patients studied, is expected in a neurogenic atrophy with only moderate muscular wasting. Increased spinal fluid protein has been stressed as an important diagnostic sign (3). If present, it supports a diagnosis of polyneuropathy but a normal value does not exclude the diagnosis, as this was found in half of the patients in whom the spinal fluid was examined.

Histological examination of a muscle biopsy specimen revealed nothing remarkable in 3 of 6 patients studied and in the remaining 3 a neurogenic atrophy indistinguishable from that occurring in an anterior horn cells disease. Studies of *nerve biopsy specimens* were also of limited value in patients without evidence of involvement of the central nervous system they are, however necessary for a specific diagnosis, such as metachromatic leucodystrophy

Electromyography of distal muscles revealed evidence of denervation in all patients examined. Needle examination may however be difficult in young children and the picture may be indistinguishable from that produced by a disease localized to the anterior horn cells. *The conduction velocity of peripheral nerves* appears the best tool for diagnosing an affection of peripheral nerves in childhood. Borderline or slightly low values may occasionally be found in young children with severe weakness and wasting due to an anterior horn cells disease (12), but this seldom causes diagnostic difficulties. In children with an affection of the central nervous system measurement of the conduction velocity may reveal otherwise unsuspected involvement of peripheral nerves.

In the group of patients with co-existing involvement of the central nervous system the *treatment course and prognosis* varied with the type and extent of the central affection. *In the* children with no signs of involvement of the central nervous system physiotherapy was the main type of treatment, supplemented with orthopedic measures when indicated. These procedures cannot influence the disease *per se* but because of its slow progress they will considerably diminish the patient's handicap. Chronic polyneuropathy has a better prognosis than most of the primary muscle diseases and progressive spinal muscular atrophy and almost all affected individuals survive. Every step must therefore be taken to decrease as far as possible the physical handicap caused by the disease.

SUMMARY

A clinical material is presented consisting of 43 children below 17 years of age with evidence of impaired function of peripheral nerves. One case occurred in a newborn with muscular hypotonia, weakness and areflexia. Thirteen children had signs of an affection also of the central nervous system. This group included _ cases of late-infantile metachromatic leucodystrophy and cases of Friedreich's ataxia. Involvement of both central and peripheral parts of the nervous system causes a complicated clinical picture. Correct

interpretation of the findings requires complete neurological and neurophysiological examination. Such an extensive affection of the nervous system may be more common than usually supposed.

In 29 patients no signs were found of simultaneous involvement of the central nervous system. The 2 main groups were children with diabetes and children with a chronic hereditary polyneuropathy. All the diabetic children were asymptomatic. The most common clinical picture of chronic hereditary polyneuropathy in childhood was characterized by slow motor development, slow clumsy movements and weak or absent muscle reflexes. Excavated feet and kyfoscoliosis were seen in some patients. The variation of the clinical picture with age and from family to family is described. The progress of symptoms in polyneuropathy without evidence of involvement of the central nervous system is slow and seems eventually to cease. The prognosis is better than that of progressive spinal muscular atrophy and most of the primary muscle diseases. An optimistic attitude, intense physical therapy and orthopedic measures are therefore justified.

ACKNOWLEDGMENTS

Dr Gordon Millichap, Children's Memorial Hospital, Chicago, made it possible for me to examine some of the patients, including the newborn infant. Docent Arne Brun and Dr. K.-G. Henriksson performed the histopathological examinations and prepared the microphotographs.

ADDENDUM

Since this article was submitted for publication, peripheral neuropathy demonstrated histologically has been reported in 6 cases of leucodystrophy of the globoid cell type (Krabbe's disease) by Sourander & Olsson (*Nord Med, 78* 1589 1967).

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CASE REPORT

OLIGURIC GLOMERULONEPHRITIS IN AN INFANT OF FOUR MONTHS

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Despite the predominance of glomerulonephritis in the younger age groups it is rare in infants (11 14).

A case of oliguric glomerulonephritis in an infant of four months is reported. The initial symptoms were convulsions, hypertension, oliguria, haematuria and uraemia. Initially the patient received a short-term treatment with corticosteroids and was later successfully submitted to peritoneal dialysis.

CASE REPORT

I. A. (Record no. KASGe 220266/66) was girl of four months with no family history of kidney diseases. She was born at term on February 22nd, 1966, after an uneventful pregnancy Weight 3600, length 53 cm. She had developed normally without intercurrent diseases and especially without noted upper respiratory tract infections.

After few vomiting spells during the preceding 24 hours she had seizure of universal tonic-clonic convulsions on June 25th, 1966. An injection of phenobarbital 50 mg was given in the emergency room and subsequently the patient was transferred to the paediatric department.

On arrival she was shivering and disquiet without fever The spinal fluid was normal 10 hours later the patient had recurrent attacks of convulsions, and an acute encephalitis was suspected Treatment with cortisol (Actocortin®) 50 mg intramuscularly cortisone acetate 25 mg t ice day and oxytetracycline (Terramycin®) 40 mg twice day intramuscularly was therefore instituted. She was now dyspnoeic, oedematous and showed metabolic acidosis of moderate degree (base excess −11 mEq/l). Sodium bicarbonate 13 mEq was therefore given orally during the following 8 hours.

On June 26th the serum potassium concentration was 8.2 mEq/l. The serum calcium concentration was 5.5 mg per 100 ml and the serum phosphate concentration was 13 mg per 100 ml. The blood urea concentration was 136 mg per 100 ml and the systolic blood pressure was 115 mm Hg. During the first 8 hours of that day she produced 20 ml of dark brown, sterile urine that was found to contain numerous white cells and erythrocytes but no casts.

Intravenous administration of insulin, 2 i.u., and glucose 50 per cent, 20 ml, caused decrease of the serum potassium concentration to 5.0 mEq/l. Calcium chloride, 3 g, was given orally without affecting the serum calcium concentration.

The patient became increasingly lethargic, the oliguria persisted and the weight increased steadily On June 28th the patient was universally oedematous with serum sodium concentration of 119 mEq/l and blood urea concentration of 288 mg per 100 ml. The suggestive diagnosis was acute glomerulonephritis but an inborn malformation of the urinary tract had to be ruled out. T gain time for necessary urologic investigations peritoneal dialysis was performed during the next 24 hours. The clinical state improved considerably and weight loss of 710 g ensued. The serum electrolyte concentrations approached the normal limits as it appears from Fig. 1.

June 29th cystoscopy was performed (T Gertz, M.D.) and the bladder was found normal. However tubulation of the ureters was not possible though the ureteral openings looked normal An exploratory laparotomy was therefore performed immediately The lower urinary tract as normal hile the retroperitoneal structures as well as the firmly enlarged kidneys showed pronounced oedema.

A peroperative kidney biopsy was performed technically the result was unfortunately not optimal The biopsy was evaluated by K. Schoettup, M D (Glostrup Hospital) the few glomeruli present show necrosis of the vascular tuft, increased thickness of the basal membranes, synechies and in some places duplication of the epithelial cells without overt crescent formation. Cellular in-

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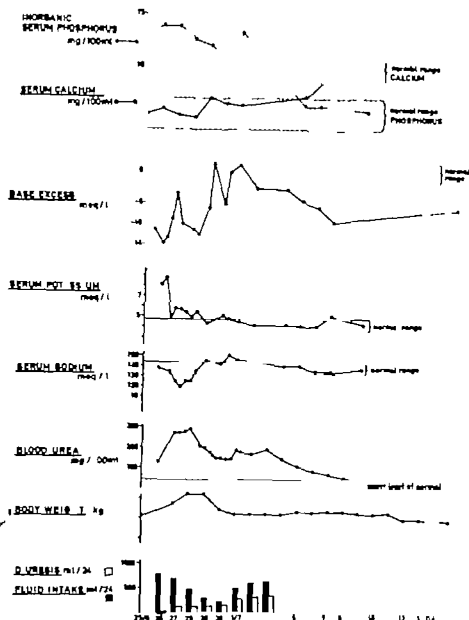


Fig 2. Fluid balance and laboratory results for the time: 25.vi.–10.vii. 1964.

filtration with granulocytes is absent, vascular changes outside the glomeruli is not seen and the interstitial tissues are normal. *Diagnosis*: presumably glomerulonephritis (no findings contradict the diagnosis glomerulonephritis but the technical state of the tiny biopsy specimen requires slight reservation).

During the following days the urine production increased, the blood urea concentration dropped and also the other laboratory values slowly became normal. The blood urea concentration was for the first time normal on July 14th, initially during the diuretic phase per sistent proteinuria was found. Because of hypoproteinaemia infusions of human albumin (totally 18 grams) were given on June 30th and on July 1st and 2nd.

During recovery passing pyuria—probably caused by the indwelling catheter—was treated with sulfamethoxy pyridazinum (Lederkyn®) and chloramphenicol palmitas (Chloromycetin palmitate®).

On August 12th the patient was discharged from the hospital in good health with slight proteinuria as the only pathological finding.

Blood titers of antistreptolysin and antistreptococ hyaluronidase were normal throughout the hospital stay.

5 weeks later the patient was readmitted because of bilateral otitis media. 2–3 white cells were found per high power field in the urine and the urine culture grew strain of b. proteus. Systolic blood pressure was 110 mm Hg and the blood urea concentration was 17 mg per 100 ml. Clinical and laboratory investigations were otherwise unremarkable. Treatment with G-penicil-



Fig. 2. Microphotographs of peroperative kidney biopsy 30 1966

lin and sulfamethoxypyridazine (Lederkyn®) was given for 10 days and the patient was discharged with slight but constant proteinuria. Long-term treatment with phenoxymethylpenicillinpotassium (Fenoxcillin®) 200,000 i.u. daily was prescribed.

January 1967 the patient was admitted for control examination. Clinically the patient was well and had developed normally. Urinalysis was normal as were the blood urea and the serum creatinine concentrations (33 and 0.4 mg per 100 ml, respectively). The urea clearance was 23 ml per minute (uncorrected, body weight 10 kg). May 1967 systolic blood pressure was 95–100 mm Hg. The urine was normal apart from the periodical content of trace amounts of protein.

COMMENT

Since 1927 a total of 43 cases of glomerulonephritis in children below 1 year's age has been reported (for survey see 3, 4, 8). However clinical and pathoanatomical informations are relatively limited in some of the older papers and the figure of 43 might be a maximum value.

Two of the patients described have died shortly after birth (10 and 90 hours post partum) and the autopsy has shown renal changes normally considered to be of a chronic nature (crescents, hyalinization of the glomeruli) (3, 4). Even despite the fact that the streptococcal infection that may bear an aethiological relationship to the glomerulonephritis in more than half of the cases is within 8 days before the acute onset of the disease (1) everything points to accept the idea of disease that in these cases have arisen in the fetal lif

The clinical diagnosis of an acute glomerulonephritis seems justified in our case because of the acute onset of convulsions, hypertension, oedemas, oliguria, and haematuria, and was supported by the macroscopic appearance of the enlarged and oedematous kidneys. No information concerning urinalysis before the acute episode was available. The microscopic appearance of the renal tissue gives a diagnosis of glomerulonephritis but the adjective acute seems hardly justified. No cellular infiltration, characteristic of the acute phase, was demonstrated, and the changes of the basal membranes, the synecchies and the epithelial duplications likewise do not point to an acute glomerulonephritis. However it is difficult to estimate the duration of the illness on the basis of these findings, severe glomerular changes (adhesions and crescents) have been demonstrated as early as 12–16 days after the appearance of the first clinical symptoms of glomerulonephritis (2).

A haemolytic-uraemic syndrome could be excluded as no signs of haemolysis were present, and the pathoanatomical laesions were incompatible with diagnosis of renal, glomerular dysplasia.

No diagnostic clue i offered by the blood antistreptolysin level as it has been demonstrated that streptococcal antibodies may pass the placenta and can be found in the blood of the newborn without apparent infection of the child (9). It

has also been found that the nephritogenic effects of the streptococci are not necessarily adherent to the haemolytic properties (12).

The *initial 3* days treatment with steroids—because of a suspicion of encephalitis—cannot be exonerated to have influenced the course of the disease. However the effect is highly difficult to evaluate as the disease may follow a capricious course, and spontaneous recovery from acute oliguric glomerulonephritis may result even after an oliguric period of 6 weeks (5). This is further supported by the observations of the Cleveland group (10) who did not reach any definite conclusions after giving treatment with steroids for one month to 9 patients with acute oliguric glomerulonephritis. This treatment did not seem to influence the course in cases of chronic glomerulonephritis *with oliguria*.

During the period of oliguria the patient may succumb from a variety of causes, for instance *overhydration, hypertension cardiac insufficiency* and cerebral oedema and haemorrhage. If the patient survives the dangerous period of uraemia and oliguria these symptoms alone will not necessarily indicate a bad long-term prognosis (1 7) Dialyzing procedures are therefore well indicated in the acute oliguric phase of a glomerulonephritis. The type of dialysis procedure may vary from one place to another depending on the experiences of the department, but generally the peritoneal dialysis tends to present fewer problems in small individuals than does haemodialysis. The procedure may be carried out in a non-specialized department as described.

Technics and possible complications to peritoneal dialysis in infants have repeatedly been described during recent years (6 13) and need no further comment here.

SUMMARY

A case of oliguric glomerulonephritis in an infant of 4 months is described.

The patient was initially treated with steroids during 3 days. When the diagnosis glomerulonephritis had been settled, the treatment comprised fluid restriction and peritoneal dialysis.

The patient was discharged after 6 weeks with slight proteinuria, a serum creatinine concentration of 0.5 mg per 100 ml and a systolic blood pressure of 90 mm Hg

Diagnosis and treatment is discussed. It is concluded that treatment with dialysis is well indicated in cases of acute oliguric glomerulonephritis while the beneficial effects of steroids is more doubtful.

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CASE REPORT

NORMAL/TRISOMY C MOSAICISM IN THE MOTHER OF A "MONGOLOID" CHILD

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As the risk of having a "mongol" child (Down's syndrome) is highly dependent upon maternal age (6), the young mother (under 30 years of age) with an affected child presents a problem in genetic counselling. Under the assumption that maternal ageing was expressed as trisomy in the affected child, translocations may be more frequently present in affected children born to young mothers who themselves may be balanced translocation carriers (3).

The present case report deals with the chromosomal findings in a young mother and her mongoloid child both of whom were investigated as a routine procedure due to the mother being under 30 years of age.

MATERIALS AND METHODS

Blood leucocyte and skin fibroblast cultures were set up from mother and child by the methods described by Bishun *et al* (1) and (2). All karyotypes were constructed following the principles of the London Conference (4).

CASE REPORT

The one year old affected/male child showed all the clinical signs of Down syndrome. He is the third child in a sibship of three, the other two children being alive and phenotypically normal.

The 27 year old phenotypically normal mother had had one spontaneous abortion and three uncomplicated pregnancies, at the last of which the affected child was born. There is no consanguinity with her normal spouse aged 30 years. Her own family history is non-contributory

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RESULTS

Sex chromatin was normal in the three children and the mother

Chromosome counts for the mother and affected child are summarised in Table 1 The chromosomes of the two other children, one male and one female, were normal. One hundred cells were examined from the skin and blood culture from the mother Thirty (30%) of these cells had a constant extra chromosome in Group C. No significance was attached to the cell with 48, 45 and 44 chromosomes since these did not show any constant chromosomal pattern. Fifteen karyotypes were constructed from the mother's cultures and they all showed a 46/XX/47/XX trisomy C mosaicism.

Ten karyotypes from the cultures of the mongoloid child established a standard 47/XY trisomy 21 chromosome constitution in both skin and blood.

DISCUSSION

The recent studies by Gianelli *et al* (5) and Polani *et al* (7) demonstrated that mongols born to young mothers, show high incidence of *de novo* translocations (7). However it was also evident that the young mothers were balanced translocation carriers in a high percentage of cases.

In view of this all mongols born to mothers under 30 years of age should be examined routinely in order to ascertain chromosomal constitutions and further elements of risks of having abnormal children

The present case of normal/trisomy C in this

Table 1 *Chromosome counts in mother and affected child*

Tissue	Chromosome counts <44	45	46	47	48	Total
Mother						
Skin	2	1	36	10	1	50
Blood (P B)	1	1	28	20		50
Total cells for the Mother	3	2	64	30	1	100
Mongol child						
Skin	—	—	1	14	—	15
Blood	—	—	1	49	—	50
Total cells for the child			2	63	—	65

mother is the first of its kind to be reported and is intriguing since she has already given birth to three live children. However it is possible that her gametogenic tissues are involved in the mosaicism which exists in her skin and blood cells, because she had had one previous abortion. Unfortunately it was not possible to culture any tissues from the abortus.

Further follow up chromosomal studies will be undertaken on any future children that may be born to this mother

SUMMARY

Chromosomal studies in a young mother (under 30 years of age) of a "mongol" child revealed a normal/trisomy C mosaicism in both skin and blood cultures. This mother has given birth to three live children, including the "mongol" child

This is the first report of such a finding in a young mother of a "mongol"

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CASE REPORT

EOSINOPHILIC LEUKEMIA — AN IMMUNOPATHOLOGICAL REACTION?

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An eosinophilic reaction is a not uncommon hematopoietic response in acute and chronic diseases. A reaction comprising the major part of all reticuloendothelial tissue is much less usual. Equally rare are those cases which have been described as eosinophilic leukemia, a disease conception whose justification and classification have been questioned and discussed from various aspects (1–14).

The object of this paper is to report another case in addition to the few previously described which fulfilled the criteria of leukemia (9–12). The present case is unique in view of the development of extremely high serological antibody titers to pneumolysin and Toxoplasma. The changes in blood and serum started with a phase characterized by a marked increase in the mononuclear leukocytes. Autopsy disclosed a large tumor of the thymus, with metastasis to lymph nodes and liver. The pathogenesis is discussed from the viewpoint of modern immunopathology.

CASE REPORT

History and Course. The patient was 5-year-old boy previously healthy apart from scarlet fever and measles. H father had a history of rheumatoid arthritis, otherwise, the family history was non-contributory. Two weeks before hospitalization, the patient had slight pharyngitis, during the first eek he was afebrile and in good condition. His cervical lymph nodes then became swollen, and his temperature rose slightly. During the third week he lymph nodes increased in size, and exanthem appeared. Examination on admission to hospital showed greatly swollen tonsils, covered by bluish grey membranes. The cervical lymph nodes were goose-egg sized hard and non-tender. Similar but smaller lymph nodes were palpable in the neck, axillae and groins. The liver was palpable 4 cm below the costal margin, and the spleen 2 cm below it. Abdominal pain was present, as well as tenderness in the region of the spleen. From the fifth week onward, petechial hemorrhages appeared over the whole body. There was increasing ascites from the sixth week. His temperature rose from the third week and reached 39–40°C in the sixth week. Thereafter it dropped lytically the boy condition slowly exacerbated, and he died after months illness.

Laboratory Data. On admission, the hemoglobin as 14.4 g/100 ml. Despite repeated blood transfusions, it fell to 8 g/100 ml, with hematocrit of 22%. Platelets 122,000/mm³ falling to 36,000 in the seventh week. Bleeding and clotting times were normal. WBC 13,200/mm³ on admission, rising to 54,000 with neutrophilic band forms 1 3%, neutrophilic polymorphonuclears 11%, rising to 64%, eosinophils 3% on admission, rising to 45% in the sixth week, thereafter falling, ordinary lymphocytes 48% in the second week and 74% in the third eek, thereafter decreasing, monocytes 5–8%. Atypical lymphocytes increased in number and reached maximum of 35% in the fifth week thereafter they fell, with value of 2% in the eighth week. Thus, the incidence of poly- and mononuclear cells as fairly normal up to the fourth week, when shift occurred to dominance of polynuclears, terminally these cells comprised 89% of the total WBC.

On admission, serum electrophoresis showed normal values; thereafter there was progressive hypoalbuminemia and slight hypogammaglobulinemia. Liver function tests: increasing bilirubinemia (to 21.0 mg/100 ml in the eighth week) and rise in SGPT from 65 to 125 units. Thymol turbidity and serum alkaline phosphatase were normal, as was the Takata reaction. Repeated cultures from the nasopharynx were negative. Serological reactions: the antipneumolysin titer was 1 1600 on admission, as well as in th fourth and fifth eeks of the illness, but rose in the eighth week to 1 17,400. The complement fixation test for toxoplasmosis rose from 1 30 to 1 120 and finally to 1 480 the dye test was 1 250, 1 1250 and 1 6250. The antistreptolysin and antistaphylolysin titers were determined several times; they were thin the normal range and remained unchanged. Widal reaction negative. The Paul-Bunnell test was negative on three occasions. Agglutination and complement fixation tests for

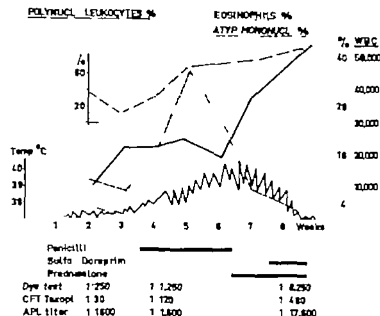


Fig. 1. Salient clinical and laboratory data.

Listeria were negative. Skin tests and serological tests for trichinosis and echinococcosis were negative. Complement fixation tests for various viruses in acute and convalescent serum failed to show any significant rise in titer. Repeated examinations of the stools disclosed no ova, helminths or other parasites.

Bone marrow aspiration in the fifth week showed "normal erythropoiesis, hypoplastic megakaryocytes, and almost total dominance of the eosinophilic series in the ... lesis (Dr J Zajicek). Aspiration of ... l lymph node in the same week disclosed firm con... ...cy a moderate number of lymphatic cells and numerous eosinophilic granulocytes.

Repeated recordings of the electroencephalogram showed grossly abnormal activity from all regions, maximal in the occipital region. The ECG was normal.

Inoculation of ascites fluid into guinea-pigs immediately after death disclosed no Toxoplasma gondii.

Postmortem Findings (Dr. H. Nordenstam). An almost fist-sized tumor with central necrosis was present at the site of the thymus. Higher up in the mediastinum, there were walnut-sized tumours, with the appearance of metastases. The liver was massively enlarged, and contained several dark brownish-red "metastases" the size of golf-balls. The preliminary diagnosis was thymoma or malignant teratoma. The lungs were infiltrated with bronchopneumonia-like lesions, similar to those seen in some virus diseases.

Microscopical examination of the thymus disclosed no typical thymus tissue, but a loose connective-tissue structure, moderately vascularized, and with scanty collagen. It was infiltrated by immature myelocytes, many of which were markedly immature; almost all of these were of eosinophilic type. A large number of mature eosinophilic granulocytes were also present. Only isolated islets of epithelioid cells suggestive of thymus tissue could be detected in this fairly homogeneous mass of myeloid cell

In two lymph nodes examined, the normal cortex-medulla structure was distinctly preserved. The reticulo-endothelium was moderately swollen. There was only a scanty incidence of slightly immature myeloid cells, mainly eosinophilic leukocytes in varying degrees of maturation. In another lymph node, the cortex-medulla structure was masked by an intense infiltration of myeloid cells of the same appearance as in the thymus.

Dense infiltrations of immature myeloid cells, mainly leukocytes of varying maturity were also present in the spleen, and interstitially in the kidneys, heart muscle, liver and lungs. Small foci of necrosis were observed in some of the myocardial infiltrates. No true broncho-pneumonia was present. *The formations in the liver* which had the appearance of metastases at gross examination, proved to be large areas of necrosis with hemorrhages.

The bone marrow of the vertebrae was highly cellular with predominance of immature leukocytes. A small number of them were markedly immature; the greater number were moderately immature, with eosinophilic granulation. The numerous eosinophilic cells were dominant throughout the bone marrow. Erythropoiesis was barely detectable, and only isolated megaloblasts were visible.

The histological diagnosis was myeloid leukemia of eosinophilic type.

To sum up, a 5-year-old boy with an essentially non-contributory history had an upper respiratory tract infection, and was admitted to hospital 2 weeks after its onset for suspected infectious mononucleosis. He had greatly enlarged superficial lymph nodes, hepatosplenomegaly and leukocytosis, with a normal distribution of poly-

nuclear and mononuclear cells. From the third to fourth week of the illness, leukocytosis progressed, with a marked rise first in the number of atypical lymphocytes, and then in the number of eosinophilic leukocytes. Terminally there was a fall in number of both types of cell. Serological examinations showed a highly marked rise in toxoplasmosis and antipneumolysin titers. At autopsy the thymus was found to be massively enlarged, with microscopical infiltrates of markedly or moderately immature eosinophilic cells, only traces of thymus-like cells case in addition to the few previously described persisted. Similar infiltrates of eosinophilic leukocytes were present in the lymph nodes, spleen, liver lungs, kidneys and heart muscle. Immature eosinophilic leukocytes also dominated in the bone marrow

COMMENT

Since the present case fulfilled the criteria for a diagnosis of eosinophilic leukemia—i.e., an abnormally large number of immature cells in the peripheral blood and/or bone marrow—it should not be classified as an eosinophilic leukemoid reaction only (3 6, 9 10, 11).

Both Bentley *et al* (9) and Odeberg (12) made detailed surveys of all cases that have been reported as eosinophilic leukemia. For all essentials, I refer to their statements and discussions of diagnosis and systematics. Bentley *et al.* were unable to verify more than nine cases before 1961 that could be classified as eosinophilic leukemia of acute blast type. In 1965 Odeberg stated that not single well-documented case of uncomplicated eosinophilic leukemia has been described.

The benign, familial, chronic eosinophilic diseases (2, 12, 13 14) which have been reported under the same heading or similar ones seem to have a completely different etiology and pathogenesis to the case described here which initially presented as a fulminating, rapidly fatal, acute granulocytic leukemia. Apparently there are several, widely disparate diseases that have been described as eosinophilic leukemias

Recently Gruenwald *et al.* (15) demonstrated, in a case of eosinophilic leukemia, the Philadelphia 1 chromosome that is known to be present in almost every case of chronic granulocytic leukemia. They also demonstrated hyperdiploidy with an extra chromosome in the C series, encountered in relation to a clinical and morphological picture of acute leukemia ("blast crises"). They concluded that eosinophilic leukemia may in some cases be a variant of the much more common (neutrophilic) form of chronic granulocytic leukemia On the other hand, Bentley *et al* (9) described some trends that should characterize eosinophilic leukemia, i.e., a usually much shorter course, transient infiltrations in the lungs, and myocardial involvement frequently leading to the development of mural thrombi. Some authors have described morphological characteristics of the eosinophilic cells differing from those of the ordinary eosinophilic leukocytes (10). Whether or not eosinophilic leukemia should be regarded as a separate entity is still a controversial question

Although, in the present case, the leukocytes exhibited morphological changes justifying a diagnosis of leukemia, certain features argue in favor of a leukemoid reaction on the basis of one or more infectious diseases. Thus, a marked rapid increase in both the total and relative number of atypical mononuclear leukocytes started in the middle of the illness. Concurrently the eosinophilic leukocytes disappeared from the peripheral blood, but this was followed by a rapid, pronounced increase in both their total and relative number This increase was accompanied by marked serological changes, in the form of a rise in the antipneumolysin and Toxoplasma titers.

It is difficult to explain the serological changes. One possibility is that the atypical mononuclear cells (interpreted at routine examination as lymphocytes) represented the immunological response to an acute (infectious?) irritant effect. After a short latency—but otherwise parallel in time—a hyperergic process started, and was reflected by change in the eosinophilic leukocytes. The initial disappearance of the eosinophilic cells is compatible with the conditions on provocation experiments in filariasis Thus, after a dose of diethylcarbamazine, the eosinophils initially decrease (shock effect), with a subsequent marked increase in the peripheral blood (blood flukes die, and *non-human protein is liberated*)

The rise in antipneumolysin titer was so pronounced that one is doubtful about the specificity of the reaction. Repeated cultures failed to disclose any pneumococci, and no pneumonia wa detectable at utopsy Nor was there any explanation of the marked serological reactions to Toxo-

plasma, culture of the ascites fluid was negative, and autopsy revealed no signs of past or present Toxoplasma infection. Theoretically the rise in titer could be explained as a disturbance in the premunition of a latent Toxoplasma infection that had initiated other abnormal antigen-antibody reactions. It seems more plausible to conclude *that the Toxoplasma reactions* were expressions of another antigen stimulus than Toxoplasma. In the dye test, cross-reactions to other *Protozoa* are common, but they are not known to occur in the complement fixation test (16).

Engfeldt & Zetterström (5) suggested that eosinophilic leukemia might be of hyperergic origin. They observed multifocal interstitial tissue degeneration and necrosis, as well as endothelial damage and capillary thrombi, with predominantly eosinophilic infiltrations. They expressed the view that the disease is of auto-immune origin, and related to Löffler's syndrome and periarteritis nodosa, and therefore proposed the term disseminated eosinophilic collagen disease This proposal has subsequently been discussed and adopted by several other authors (6, 8, 12, 13 14)

Recently Pierce *et al* (13) have reported a case followed during more than a year similar to the patients described by Engfeldt & Zetterström and Bousser (7). The essential changes were found the skin, lungs, heart, liver and kidneys, but there was no splenomegaly and a conspicious lack of adenopathy Vasculitis and capillary thrombosis were found postmortem. The bone marrow was hypercellular with granulocytic hypoplasia *and immaturity with eosinophilia consistent* with chronic myelogenous leukemia. Pierce *et al* discuss the eosinophilia as a response to healing of vasculitis, "to environmental toxicities and hyperimmunity perhaps to drugs, bacterial products, or sustained autoantigenic stimuli

A comparison between the features in the cases previously reported and in the present one is of interest, particularly with respect to the postmortem findings in the thymus, and their relation to the immunological reactions. In genuine leukemia, the antibody-producing capacity is generally decreased and has never been observed in leukemic cells' consequently the serological reactions to various infectious antigens usually weaken or disappear For this reason, high serological titers argue, *a priori*, against genuine leukemia. On the other hand, an enormous production of globulin is a characteristic feature of myeloma, and it is presumed that previously antibody-producing cells proliferate to tumor cells in this process, although the inducing antigen is unknown. According to Burnet's theory (17 18, 19), the thymus plays a key role in the immunopathological process, by *regulating the homeostatic control in the* antibody-producing system by eliminating the antibody producing cells which are directed against one's own antigens, i.e., which are included in the forbidden clones

This theory might support the present case being explained on the basis of changes in the controlling organ—the thymus—giving free scope for neoplastic proliferation of certain antibody producing clones (reflected in the serological titers for pneumococci and Toxoplasma)

The leukemic infiltrates may have been caused by destruction of the homeostatic control by an unknown factor or they may have been induced by a foreign infectious antigen. After about three weeks—possibly in a delayed reaction process—this antigen may have elicited an abnormal immunological reaction—sharing the determinants with endogenous tissue—which was followed by a hyperergic phase with eosinophilic proliferation In this phase, the ability of the thymus to balance the homeostatic control had presumably ceased.

It nevertheless remains difficult to explain how the cells create antibodies to precisely two and highly different antigens. Another way of approaching the problem is to presume that antibody-producing cells proliferate in the absence of *a controlling mechanism. Normally however* the antibodies inhibit their own production after some latency Some disturbance of the normal inhibition mechanism in one or two clones in connection with cessation of thymic control might afford the possibility of neoplastic proliferation.

Challenging various hypotheses on the ways in which auto-immunization can occur the WHO Scientific Groups on General and Applied Research have discussed the possibility that autoantibodies are produced by immunization with *microorganisms which share antigenic determinants* with tissue antigens, and that the immune response to the foreign antigens also includes the shared antigenic determinants (20).

The concepts of modern immunopathology seem to support the conclusion that, in the present

case, the thymus played an important role both in the suggested neoplastic proliferation of antibody-producing clones, and in the proliferation of eosinophilic leukocytes. The question of whether or not eosinophilic leukemia is a separate entity appears to be of subordinate importance.

SUMMARY

An account is given of eosinophilic leukemia in a 5-year-old boy who died after 2 months illness. Post mortem examination disclosed a large tumor in the thymic region, as well as infiltrates of eosinophilic leukocytes, mainly immature, in the thymus, lymph nodes, spleen, lungs, heart and kidneys.

After 3 weeks illness, the number of atypical mononuclear leukocytes in the peripheral blood increased markedly. This was followed by an increase in the number of eosinophilic leukocytes. Concurrently a pronounced rise took place in the serological titers for antipneumolysin and Toxoplasma.

The mechanism of development of the changes is discussed gainst the background of a suggested immunopathological process, in which the thymus may have played central role.

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CASE REPORT

CONGENITAL ADRENAL HYPOPLASIA IN TWO INFANTS

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From the Institute of Obstetrics & Gynaecology Hammersmith Hospital London, Great Britain

Cases of congenital adrenal hypoplasia in neonates and infants are extremely rare. One of the earliest specimens was described by Sikl (7) in an infant aged 33 days, and 3 further cases of this condition were mentioned by Potter (5) Roselli & Barbosa (6) found only 23 cases of newborns with necropsies reported in the literature and added _ of their own. They suggest as other writers before them (2), that cases of this condition may have been overlooked in the past. We have also ly had this experience on reviewing some our cases.

According to Symington (8) two different types of congenital adrenal hypoplasia may now be histologically recognised, namely the anencephalic and the cytomegalic type. The anencephalic variety where the adrenal gland is small and consists almost entirely of provisional or adult cortex, does not show evidence of any foetal zone. This appearance is also seen in anencephalic and cyclopic foetuses. Symington states that it may result from lesions of the cerebral cortex, pituitary and hypothalamus, although this is not always the case and, therefore the aetiology is unknown. It is also the type of gland which occurs when the mother during pregnancy has been treated with the newer steroid preparations which cross the placental barrier These babies usually die within the first ten days of life. In the cytomegalic type of congenital adrenal hypoplasia, the whole of the very narrow cortex is composed of rather large cells with eosinophilic cytoplasm which resemble the cells of the transient cortex but are irregular in size and arrangement and often vacuolated. In some instances there is evidence of adrenocortical insufficiency such as pigmentation of skin and electrolytic disturbances. In this variety of congenital adrenal hypoplasia, which may be familial, the infants may survive longer than in the anencephalic type. The condition is assumed to be a failure or arrest of development rather than de generation or atrophy (3)

During a large scale investigation on the influence of various conditions on certain reproductive organs of the foetus and infant (12) we have also encountered _ new cases of congenital adrenal hypoplasia which were examples of the true anencephalic and cytomegalic type and which have not previously been described.

CASE REPORTS

Case I (Baby R R.)

A first-born male infant weighing .380 g was born on 24 2 1962. The pregnancy and delivery had been normal but the mother aged 22 years, developed puerperal psychosis. The infant took its feeds quite well during the first month of life, but he had frequent stools and failed to gain weight. Thereafter he did not take his feeds ell, the stools became more frequent and were of greenish loose consistency and he lost weight. On admission to hospital (4 4 62) he was small emaciated baby and had hypothermia. H was moderately dehydrated and the fontanelle was depressed. There was rather weak feeble cry No obvious abnormalities were found in any system. He was treated ith Kanomycin and Tetracycline as it was felt that there was an infective ailment present H condition deteriorated and he lost eight rapidly H died despite all efforts on 10 4 62, at the age of 6 eek and 3 days. On the day of death his eight was 1890 g.



Fig. 1 Hypoplastic adrenal gland in an infant, aged 6 1/2 week (Case 1). The gland consists almost entirely of provisional or adult cortex. There is no evidence of any foetal zone. The adult cortex shows differentiation between zona glomerulosa and zona fasciculata. The medullary tissue is comparatively abundant. *A*, Zona glomerulosa, *B* zona fasciculata, *E* medullary tissue *V* central vein (Haematoxylin and Eosin 100)

Fig. 2. Hypoplastic adrenal gland in neonate aged 27 days (Case 2). The gland consists of extremely enlarged compact cells. They are pleomorphic and some cells contain giant nuclei with one or even two prominent nucleoli. There is hardly any medullary tissue *V* central vein (Haematoxylin and Eosin 100)

Fig. 3. Normal adrenal gland in an infant, aged 6 weeks. Control. The permanent adrenal cortex is already well differentiated into zona glomerulosa, zona fasciculata and zona reticularis. The foetal cortex is still present but there is hardly any medullary tissue. *A*, Zona glomerulosa, *B* zona fasciculata, *C* zona reticularis, *D* foetal cortex, *V* central vein (Haematoxylin and Eosin 100)

Necropsy. The body did not show any external congenital abnormalities. On internal examination, the lungs showed some patchy collapse of the lower lobes. No significant pulmonary infarction was present. The heart, great vessels and other cervical and thoracic organs were normal. No abnormalities were detected in the abdominal and pelvic organs. Skull and meninges showed some congestion of the pial vessels and some old haemorrhage in both choroid plexus. There was no free haemorrhage in the ventricles. No abnormalities of the endocrine glands were reported, but both adrenals were very small and showed an extremely thin cortical layer. Their combined weight was 1.2 g (normal average weight at this age according to Tähkä (10) is 4.30 g ± 0.34).

Our own findings

Histology. Adrenal glands. The gland consist essentially of provisional or adult cortex. There is no evidence of any foetal zone (Figs. 1 and 3). The adult cortex shows differentiation between zona glomerulosa and zona fasciculata. The medullary tissue is comparatively abundant.

Testes. Both testes were smaller than normal controls, but showed a normal histological picture.

Epididymides. The epididymides were normally developed and showed a normal histological picture. They had the ... secretory activity usually seen at that age (1).

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CASE REPORT

CONGENITAL ADRENAL HYPOPLASIA IN TWO INFANTS

Lilly H Zondek and Theodor Zondek

From the Institute of Obstetrics & Gynaecology Hammersmith Hospital London, Great Britain

Cases of congenital adrenal hypoplasia in neonates and infants are extremely rare. One of the earliest specimens was described by Sikl (7) in an infant aged 33 days, and 3 further cases of this condition were mentioned by Potter (5). Roselli & Barbosa (6) found only 23 cases of newborns with necropsies reported in the literature and added 2 of their own. They suggest as other writers before them (?) that cases of this condition may have been overlooked in the past. We have also recently had this experience on reviewing some of our cases.

According to Symington (8) two different types of congenital adrenal hypoplasia may now be histologically recognised, namely the anencephalic and the cytomegalic type. The anencephalic variety where the adrenal gland is small and consists almost entirely of provisional or adult cortex, does not show evidence of any foetal zone. This appearance is also seen in anencephalic and cyclopic foetuses. Symington states that it may result from lesions of the cerebral cortex, pituitary and hypothalamus, although this is not always the case and, therefore, the aetiology is unknown. It is also the type of gland which occurs when the mother during pregnancy has been treated with the newer steroid preparations which cross the placental barrier. These babies usually die within the first ten days of life. In the cytomegalic type of congenital adrenal hypoplasia, the whole of the very narrow cortex is composed of rather large cells with eosinophilic cytoplasm which resemble the cells of the transient cortex but are irregular in size and arrangement and often vacuolated. In some instances there is evidence of adrenocortical insufficiency such as pigmentation of skin and electrolytic disturbances. In this variety of congenital adrenal hypoplasia, which may be familial, the infants may survive longer than in the anencephalic type. The condition is assumed to be a failure or arrest of development rather than degeneration or atrophy (3).

During a large scale investigation on the influence of various conditions on certain reproductive organs of the foetus and infant (12) we have also encountered 2 new cases of congenital adrenal hypoplasia which were examples of the true anencephalic and cytomegalic type and which have not previously been described.

CASE REPORTS

Case I (Baby R. R.)

A first-born male infant weighing ..380 g was born on 24.2.196.. The pregnancy and delivery had been normal but the mother aged 2.. years, developed puerperal psychosis. The infant took a feeds quite well during the first month of life, but he had frequent stools and failed to gain weight. Thereafter he did not take his feeds well, the stools became more frequent and ... of greenish loose consistency and he lost weight. On admission to hospital (4.4.62) he was a small emaciated baby and had hypothermia. He was moderately dehydrated and the fontanelle was depressed. There was a rather weak feeble cry. No obvious abnormalities were found in any system. He was treated with Kanamycin and Tetracycline as it was felt that there was an infective ailment present. His condition deteriorated and he lost weight rapidly. He died despite all efforts on 10.4.6.., at the age of 6 weeks and 3 days. On the day of death his weight was 1890 g.

small. The organs were represented by slight thickening in the renal fascia. Nowhere are the glands thicker than 1 mm. A distinct suprarenal artery could not be identified. On section, lipoid were not seen.

Our own findings

Histology of adrenal glands. The permanent cortex cannot be differentiated. The glands consist almost entirely of extremely enlarged eosinophilic compact cells. They are pleomorphic and some cells contain giant nuclei with one or even two prominent nucleoli. There is hardly any medullary tissue (Figs 2 and 3).

Testes. Both testes were smaller than normal controls but showed normal histological appearance.

Epididymides. The epididymides were normally developed and showed a normal histological picture. They had the strong secretory activity usually seen at that age. An accessory nodule of adrenal cortex was attached to the head of the right epididymis. On histological examination, it shows narrow rim of adult cortex, consisting of narrow zona glomerulosa and zona fasciculata. The centre of the nodule is formed by foetal adrenal cortex, showing marked degree of vacuolisation and which composes the greater part of the nodule (Figs. 4 and 5). The appearance seems to correspond to that of an accessory adrenal gland, found in normal person.

Sections of the pituitary glands were not available in these two cases.

Further history of mothers

There was no report of steroid treatment given to the mothers during pregnancy. The mother of Case 1 had second pregnancy complicated by mild preeclamptic toxaemia in January 1967 and was delivered of normal infant.

The mother of case 2 had three further pregnancies, two of them female children who are alive and well, and one incomplete abortion.

DISCUSSION

The adrenal histology in our two infants showed extreme examples of the anencephalic type (Case 1) and of the cytomegalic type (Case 2) of congenital adrenal hypoplasia. Symington (9) suggests that the adrenal histological picture in the anencephalic type of case might be produced if the pituitary is either deficient in basophils or if ACTH is only secreted in small amounts. He postulates that the pituitary may even be normal macroscopically as in our case, and perhaps even contain its normal quota of basophil yet these cells are not producing ACTH. In future cases, it may be worth while to determine the blood ACTH, the ACTH content of the pituitaries and, if possible, to localise ACTH to the specific cells.

Infants with the anencephalic type of congenital adrenal hypoplasia usually die within the first ten days of life. No explanation can be offered why our Case 1 should have survived so long.

Our second case showed an example of the true cytomegalic type of congenital adrenal hypoplasia and the time of death (27 days) is consistent with this picture. No explanation, however, can be offered why the accessory gland developed normally whereas the true adrenals have failed to develop an adult or provisional cortex. This child had been treated with steroids, and endocrine gland involution therapy has, therefore, to be considered. However, if steroids did have any effect, it would have produced atrophy of the adult cortex and there is very little adult cortex in the cytomegalic type of congenital adrenal hypoplasia. In our Case 2, the adrenal glands were almost exclusively composed of the large cytomegalic compact cells and one can therefore conclude that the hypoplasia was present at birth and not an effect of steroid therapy. Moreover, the accessory adrenal in this case had developed normally and presented the appearance of an accessory gland which one would expect in a normal person.

The size of the testes was reduced in our 2 infants but we found the histological picture to be normal. According to Kerenyi (2), the anencephalic type of congenital adrenal hypoplasia also frequently shows hypoplasia of the thyroid and gonads. It may also be noted that in an investigation on the testis in anencephaly (13) we found marked hypogonadism in nearly all cases together with the adrenal hypoplasia which is associated with this condition.

Our 2 infants presented with gastrointestinal symptoms since birth. In Case [illegible], laparotomy was performed as mechanical obstruction was suspected because of persistent vomiting. No organic abnormality was detected. A similar case was reported by Mitchell & Rhaney (4), whereas in one of the infants, recorded by Boyd & MacDonald (1) volvulus was found on exploratory laparotomy. According to Weens & Golden (11), adrenocortical insufficiency in infants may manifest itself by severe vomiting and dehydration, simulating high intestinal obstruction. In one of their cases, the roentgenological signs of hypertrophic pyloric stenosis were demonstrated, while in the second case the roentgenological pattern of duodenal obstruction was simulated. Necropsy failed to reveal gastrointestinal lesions. There was generalised

cortical adrenal hypoplasia in one instance, and a severe destructive process in the adrenal cortex of the other case.

During the last few years, congenital adrenal hypoplasia has been described to occur in various members of a family (1 4). There was, however no family history of the disease in our 2 cases.

It is of interest to note that the mothers of our 2 cases did not have steroid treatment during pregnancy as this type of treatment may sometimes be responsible for the occurrence of the anencephalic type of congenital adrenal hypoplasia.

Diagnosis and treatment of cases of congenital adrenal hypoplasia are not discussed here. It should, however be stressed that early recognition of the condition is of great importance regarding the treatment of these infants. This is borne out by the fact that one of the two male siblings, reported by Mitchell & Rhaney (4), is still doing well on maintenance steroid therapy on which he was put over 9 years ago.

SUMMARY

Two new cases of congenital adrenal hypoplasia in male infants have been described. In one case, the adrenals showed the true anencephalic type of the disease and the infant survived for $6^1/_2$ weeks without treatment. In the second case, the adrenals showed the true cytomegalic type and the infant died at the age of 27 days after treatment with steroids. There was also an accessory adrenal gland attached to the head of the right epididymis.

The significance of these findings is discussed.

ACKNOWLEDGEMENTS

We are most grateful to Dr A D. Barlow and Dr A Kandhen (West Middlesex Hospital) for their permission to publish these cases. W are much indebted to Professor C. V Harrison (Hammersmith Hospital) for permitting us to photograph the histological section of a normal infant's adrenal gland. Our grateful thanks are due to Professor T Symington (Royal Infirmary Glasgow) who has given us most valuable advice with regard to some of the problems, involved in these cases and on the interpretation of the adrenal sections. Our thanks are also due to Mr W Brackenbury (Photographical Department, Hammersmith Hospital) for the photomicrographs.

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LETTER TO THE EDITOR

COMPOSITION OF THE SKIN IN MUCOVISCIDOSIS

From the Department of Paediatrics at Blegdamshospitalet Copenhagen, and the Department of Medicine F, Københavns Amts Sygehus, Glostrup, Denmark

We have examined the composition of the skin in children with mucoviscidosis (m.v.) in comparison with healthy children. We felt that, for several reasons, the electrolyte and mucopolysaccharide composition of the skin might be altered in this disease. The connective tissue ground substance of the skin may be said to be "inserted" between the blood plasma—which has normal electrolyte concentrations in patients with m.v.—and the sweat with its highly abnormal electrolyte contents. An abnormal concentration gradient may therefore exist across the ground substance. It might also be possible to demonstrate abnormal conditions in the skin if the ground substance were involved in the pathogenesis of the disease. Furthermore the composition of the ground substance might be unspecifically altered as a result of the disease.

The material included 10 children with m.v. and 11 healthy children. From all subjects a skin biopsy was obtained from the right or left groin under general anaesthesia. The subcutaneous fatty connective tissue was carefully removed and the analyses carried out on the isolated corium. The *water* content was determined by freeze-drying, the *fat* content by defatting of the biopsies in ether. The dry fat-free substance was analyzed for *sodium and potassium* by flame-photometry, for *hexosamine* by Elson & Morgan's method (1), for *uronic acid* by Dische's carbazole method and Brown's orcinol method (2) and for *hydroxyproline* by Neuman & Logan's method (5) (cf. also 3, 4).

In the age group 6 months to 12 years (7 children with m.v. and 7 healthy children) the children with m.v. were found to have higher percental fat content and a lower percental dry substance content than the normal children (Table 1). The hexosamine and uronic acid contents, as measures of the amount of acid mucopolysaccharides of the skin, were significantly reduced in relation to the

Table 1. *Amounts of water, fat and fat free solids in the skin expressed in per cent of the wet weight of the samples*

The values are mean values ± standard error of the mean

Number of patients	Age	Water (per cent)	Fat (per cent)	Fat-free solids (per cent)
Mucoviscidosis				
7	7 months–11 years (mean 5½ years)	6[illegible] ± 1.5	14 ± 1.5[a]	24 ± 0.4[a]
Controls				
7	2 years–10 years (mean 4½ years)	64 ± 0.8	8 ± 0.6	28 ± 0.5

[a] Significantly different from the control group at p 0.001.

Table 2. *Amounts of hexosamine, uronic acid and hydroxyproline in the skin of the same individuals as in table 1*

The values are mean values ± standard error of the mean

Hexosamine mg/100 g[a]	Uronic acid[b] mg/100 g[a] Orcinol	Uronic acid[b] mg/100 g[a] Carbazol	Hydroxyproline g/100 g[a]
Mucoviscidosis			
364 ± 28.4[c]	132 ± 2.9[c]	115 ± 7.1	8.0 ± 0.32
Controls			
44[illegible] ± 20.4	189 ± 22.9	155 ± 22.3	8.4 ± 0.20

[a] Fat-free solids.
[b] Determined in 5 patients only.
[c] Significantly different from the control group at p 0.01.

Table 3 *Amounts of sodium and potassium in the skin, expressed in mEq/100 g fat-free as well per liter tissue water*

The values are mean values ± standard error of the mean

Number of patients	N mEq/100 g fatfree solids	N mEq/l total tissue water	K mEq/100 g fatfree solids	K mEq/l total tissue water
Mucoviscidosis				
10	29 ± 1.0*	117 ± 3.5*	9.5 ± 0.68	38 ± 2.3
Controls				
11	36 ± 1.9	137 ± 5.4	9.7 ± 0.63	36 ± 1.6

Significantly different from the control group at $p < 0.01$

normal material (Table 2) Lastly the sodium concentration in the tissue was significantly reduced (Table 3).

The results clearly show that the skin of children with m.v differs from the skin of normal children. As in non-specific stress a reduction in the water content of the skin is a constant and very early finding in animal experiments, the normal water content of the skin in the m.v patients militates against the changes being non-specific sequelae. There is a relative reduction in the amount of acid mucopolysaccharides, whether these are measured in terms of hexosamine or uronic acid As the acid mucopolysaccharides of the skin, in particular hyaluronic acid and chondroitin sulphate B, bind cations, making the extracellular fluid seem hyperosmolar in relation to plasma, it is reasonable to relate this low mucopolysaccharide content to the simultaneous finding of a relatively reduced sodium content. Whereas it is speculative to assume a direct relationship between the low sodium binding capacity of the connective ground substance in m.v and the high excretion of sodium in the sweat, it seems reasonable to conclude—on the basis of the reported findings—that the connective tissue ground substance is a medium in which the disease also manifests itself

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PROCEEDINGS OF PEDIATRIC SOCIETIES

THE SWEDISH PEDIATRIC SOCIETY

Meeting March 16 1966

O Flanström: *Wiskott-Aldrich's syndrome*

Wiskott-Aldrich's syndrome which is characterised by thrombocytopenia with a tendency to bleeding, increased liability to infection (particularly bacterial infections) and eczema was first described by Wiskott in 1936. In 1956 Aldrich *et al.* demonstrated that it was a hereditary condition transmitted as a sex-linked recessive.

Two brothers, 6 months and 18 months old, with typical symptomatology were examined in the pediatric clinic at Umeå. The family history was negative. The older brother's case was characterised by a bleeding tendency the younger's by eczema and increased susceptibility to infection.

The number of thrombocytes varied between 10–150,000 The duration of bleeding was protracted, but coagulation-time was normal. Coagulation retraction was protracted and prothrombin consumption increased. Thrombocyte antibodies could not be demonstrated. The results indicate that the bleeding tendency is caused by thrombocytopenia.

Bone marrow examinations showed that the normal thrombocyte agglutination around the megakaryocytes was absent. Eosinophils were noted together with a reduced number of plasma cells.

Mantoux was negative notwithstanding BCG-vaccination. Serum electrophoresis was essentially normal. Immuno electrophoresis with quantitative determination of the various gammaglobulin fractions showed an increased IgA fraction The younger brother had a low content of isoagglutinins in serum, which is part of the picture the elder brother on the other hand, had high titers. Passive cutaneous anaphylaxis examination, with milk as antigen, was positive. Roentgenological examination showed that neither had adenoid

G Koch: *Veno-arterial shunt during the neo-natal period*

1. *Position with regard to normal newborns* The healthy newborn child has a relative arterial hypoxemia with an alveolo-arterial $(P_{AO_2} - P_{aO_2})$ P_{O_2} difference amounting to about 20–35 mm Hg. Alveolar hypoventilation as a cause of this hypoxemia can be excluded with regard to P_{CO_2} which, throughout the whole neonatal period lies below 40 mm *Hg*. Among other possible cause irregular ventilation-perfusion conditions and decreased diffusion can be eliminated by inspiration of 100% oxygen and total wash-out of nitrogen under these circumstances a remaining $P_{AO_2} - P_{aO_2}$-difference depends entirely on a true veno-arterial shunt. The localisation of such a shunt (cardiac or intrapulmonary) cannot, however be determined.

In 31 full-term infants during the first seven days of life, the oxygen tension (P_{aO_2}) was determined after 20 minutes inspiration of 100% O and the total veno-arterial shunt was calculated by means of the classical shunt formula, assuming an arterio-venous oxygen difference of 35 ml 0 7 blood. The arterial blood was obtained through a polyethylene catheter placed in the umbilical artery

From the age of 5 hours P_{aO_2} in all cases was greater than 400 mm Hg and the corresponding shunt was less than 20% of the volume per minute The average P_{aO_2} was between 500–550 mm Hg. and for the shunt between 10–15 On the first examination (30–50 minutes after birth) the P_{aO_2} average was considerably lower and the spread was 100–500 mm *Hg* for P_{aO_2} and 12–40 for the shunt The examination results thus show that t least one reason for relative hypoxemia in a

newborn is a venoarterial shunt which from the age of 5 hours probably is localised to the lungs. The method seems particularly suitable for estimation of the extent of pulmonary atelectases and of right-to-left shunt in small children in whom measurement of functional circulatory parameters is particularly difficult.

G Koch & H. Wendel. *Veno-arterial shunt during the neo-natal period*

II *Clinical application*. As from this year in cases of respiratory insufficiency in newborns we have, in addition to determining the acid base status, also carried out serial examinations of oxygen tension in the arterial blood as well as the hyperoxia test. Thus a more complete picture was obtained of the functional condition of the lungs and pulmonary circulation.

Four different types of initial respiratory difficulty were demonstrated.

1 Aspiration syndrome (shunt 50 and 85%) Died after 24 hours.
2. Very premature infant with kernicterus (normal lung function and 23% shunt) Died after one week.
Hyaline membrane syndrome (shunt 26%). Survived.
4 Serious hypovolemia and serious acidosis, with pH 7.03 (shunt 30 subsequently 20%) Survived.

In those cases where the arterial oxygen gas tension exceeds about 100 mm Hg with the hyperoxia test the prognosis seems relatively favourable. This corresponds to a shunt of less than 45% of the minute volume.

Britt Fredrikzon & S. Sjölin *Hospital meals for children*

Consumption of calories, protein, fats, carbohydrates, iron and vitamins in the diet of 30 children aged 6–16 years undergoing treatment in the pediatric clinic at Umeå was determined over a period of two days. None of the children was seriously ill or suffered from nutritional disturbances. All were out of bed during most of the day

The average caloric consumption was considerably lower than what is normal for healthy children. The low calorie consumption was probably due both to reduced physical activity and an appetite impaired by illness. On some days the unappetising nature of the food also certainly played a role. The total caloric breakdown into protein, fats and carbohydrates revealed a much higher proportion of fats than that recommended—42% as compared with the recommended 30–35%. The intake of protein, niacine, iron and vitamins A, D and C was, in most cases, considerably lower than the accepted daily requirements for healthy children. The main reason was probably low calorie consumption, but another reason was faulty composition of the diet. Also as regards the serving and tastiness of the food the study showed there was much to be desired.

Requirements for increased protein and reduced fat content can certainly easily be met by adequate changes in the diet. In order to ensure sufficient vitamin-C consumption for hospital children fruit or juice containing this vitamin should be included daily in their diet. It is unlikely that either vitamin A or D requirements can be satisfied solely through the diet, especially since the fat content must be reduced. Administration of vitamins A and D by means of medicaments would therefore appear necessary. It is doubtful whether the diet can guarantee a sufficient iron intake. Also here the hospital diet may have to be supplemented by medicaments.

Meeting May 18, 1966

K. H Gustavson, Anna-Lisa Annell & G Lyttkens: *Klinefelter's syndrome associated with chromosomal mosaicism*

The majority of men with Klinefelter's syndrome are sex-chromatin positive. The incidence of sex chromatin positivity among new-born male infants and among recruits has been reported as 1 500. Slight mental retardation is a common clinical feature among these subjects. Psychasthenia psycho-infantilism a tendency towards erratic behaviour uncertainty about sexual identity result

ing in maladjustment and difficulty in establishing interpersonal relationships, are characteristic personality traits. There is as yet no evidence that these psychological traits are present before puberty

During the last three years the sex chromatin of all male patients admitted to the Psychiatric Clinic for Children and Adolescents at the University Hospital in Uppsala, has been determined by the buccal smear method. Of 414 male patients examined 3 were found to be sex-chromatin positive the sex-chromosomal constitution being XXY XY/XXY and XY/XYY/XXYY respectively

In cases of Klinefelter's syndrome the clinical symptoms associated with mosaicism are often less pronounced than those in cases of typical XXY syndrome and may even differ from the latter. However increased height in childhood, mild dysplasia, psycho-infantilism and a tendency toward passive reactions also appear to be characteristic features of patients with mosaicism.

S. G O Johansson & Torsten Berg: *Immune globulin levels in healthy children.* (Published in *Acta Paediat Scand* 57 572, 1967)

S. G O Johansson & Lars Wranne: *Maternal indirect Coombs' titer and Rh-erythroblastosis*

During the four years between 196.. and 1965 193 Rh-immunized mothers were studied at the University Hospital, Uppsala, Sweden. Foetal and neonatal losses due to Rh-erythroblastosis included 16 stillbirths (=8%) and 4 neonatal deaths (=2.3%). Indirect Coombs titers (ICT) were determined in all mothers. At delivery 15 of the 16 mothers of stillborn babies had an ICT of 1 40 or higher The ICT titer in the 6 mothers whose babies died in utero befor the 35th week of pregnancy did not appear to be higher than in the 10 women who had an intra-uterine foetal death after 35 weeks. *Imminent foetal death did* not seem to be associated with a significant rise of titers The four neonatal deaths occurred in spontaneously-delivered infants. Three of the four mothers had an ICT of 1 40 or higher In the fourth case, death might have been due partly to non-erythroblastotic causes.

An attempt was made to correlate the ICT of the mother with the clinical course of surviving infants using the following criteria cord blood haemoglobin concentrations, rise in plasma bilirubin concentrations between birth and the first exchange transfusion, and number of transfusions made during the first 24 hours of life Most babies seemed to have mild disease if their mother's ICT was 1 20 or less.

The indirect Coombs titer of pregnant mothers does not accurately predict the severity of disease in the foetus. However titers of 1 20 or less seem to indicate that intrauterine death or severe disease of the liveborn infant is unlikely Titers of 1 20 or less were observed at delivery in 58 of all immunized mothers. In these cases, amniocentesis seems unnecessary

Kjell Bergström, Herman Lodin & Irène Sjögren. *Echoventriculography and cerebral pneumography for determination of ventricular size in children*

Fifty-six neuropaediatric patients at the University Hospital, Uppsala, in whom cerebral pneumography revealed symmetrical lateral ventricles were also subjected to ultrasonic echo examination of the ventricles. The results of the echo ventriculographic and cerebro-pneumographic examinations were compared with regard to the size of the lateral ventricles and to the widths of the third ventricle and midline echo The respective magnitudes are expressed in relation to the external cranial diameter as indices. I_{ex} and I_{pneu} denote the combined widths of the lateral ventricles in the region of the cellae mediae and I and I_m the widths of the third ventricle and the *midline echo respectively all in relation to the* external cranial diameter with the two methods of examination.

The measurements and index calculations on the echoventriculograms and pneumograms wer performed independently by two of the authors (Sjögren and Bergström, respectively) without either having any knowledge of the other's results before the final comparisons were mad

It was found that in children *over 1 year of age* with a normal intracranial pressure, echoventriculography was highly suitable for differentiation between a normal and dilated third ventricle but not for determining the sizes of the lateral ventricles or for evaluating parenchymal changes

In children of *less than 1 year* echoventriculography allowed accurate differentiation between normal and dilated ventricles, and also accurate evaluation of the size of dilated lateral ventricles (coefficient of correlation between I_{EEO} and I_{PNEUMO} = 0 85)

Meeting Sept. 10 1966

Nils Borgfors & Per Selander *The incidence of celiac disease in Sweden*

Data has been collected from all pediatric clinics in Sweden concerning celiac disease. The diagnosis was considered *definite* in those cases where the child suffered from chronic or recurrent diarrhoea, underweight or weight-loss, failure to thrive, prominent abdomen and fatty stools, and where its condition improved on a gluten-free diet and deteriorated on a diet that was normal for its age. Regard was also paid to data concerning glucose xylose and fat tolerance, trypsin- and serum protein examination, sweat test, roentgenograms of the intestine and skeleton as well as intestinal biopsy Probable celiac disease was the diagnosis ascribed to cases with clear-cut clinical findings but in which one or more important tests had not been carried out.

For the country as a whole, the incidence of *definite* cases up to 31 12.1964 among children born during the period 1950–1962 was 1 6 per 10 000 live births (1 6.500). For *definite and probable* cases the incidence was 2.1 (1 5.000) The incidence was the same in the provinces as in the larger towns.

An increased number of cases were registered during the period 1960–1963 as compared with 1950–1959 (4 0 and 1 7 respectively for definite and probable cases). No clear explanation of this has been discovered.

The incidence for the entire study period in Sweden accords with the findings in Great Britain.

H. Enell & T Wahlén. *Preliminary results of a perinatal study*

Since 1963 a longitudinal study of all pregnancies at Hälsingborg Pre-Natal Clinic has been carried out. The children were given a thorough examination at the age of one year and an exhaustive examination at the age of 4 years is planned. The study so far comprises 3500 pregnancies, resulting in 2700 births and 320 abortions. It is estimated that the total number of pregnancies will be about 5000.

The authors have studied the incidence of prematurity in relation to psycho-social status, to the pregnancy (whether desired or not), and to the occupational status of the mother The incidence of prematurity has likewise been studied in relation to infections during pregnancy especially of the urinary tract. Hitherto processed data shows that prematurity is commoner among mothers who are heavy smokers.

Agneta Lindsjö *Familial cardiac insufficiency with mors subita*

Since Evans in 1949 described some cases of familial cardiomyopathy an ever-increasing number of reports have been received concerning additional families affected by these symptoms, which are so diverse from the clinical, pathological and anatomical viewpoints. (This excludes familial myocardial diseases related to neuromuscular diseases.) In a Hälsingborg family 5 out of 7 children died with symptoms of oedema, dyspnea with exertion and hepatomegaly The symptoms appeared unusually early—between two and five years of age. In all the children the disease followed a very fluctuating course, including long periods of improvement without therapy The duration of the disease varied between 1 1/2–10 years. In one of the children—born 1951 died in spring of 1966—the initial symptoms were very mild. She died suddenly at a gymnastics lesson without having been examined previously by means of ECG and roentgenogram of the heart. Another sibling died suddenly in connection with an intestinal infection. The other three died following an increase in insufficiency symptoms. Two of the children were born with malformations—a boy with left foot missing and a girl with a markedly dysplastic uterus. ECG were taken on 4 children, all with pathological *P*-waves. There were no arrhythmias. In all cases autopsy revealed marked cardiac hypertrophy dilatation of the right atrium

and normally located but dilated venae cavae. Septal hypertrophy was not found. In two cases there were large scars in the connective tissue in the wall of the left ventricle, rather like the scars encountered following infarcts. In all cases there was diffuse myocardial fibrosis. Two cases were characterised by striking changes in the coronary vessels, with thickening of the intima, connective tissue proliferation in the media as well as splitting and degeneration of the elastic fibres. No abnormal glycogen deposits could be demonstrated, and there was no evidence of fibroelastosis or Fiedler's myocarditis.

B. Palmgren. *Some syndromes with diencephalic obesity*

A diencephalic injury may through involvement of various neuro-hormonal centres, give rise to highly diverse symptomatology.

A case of so-called Russell's Emaciation syndrome shows that involvement of this area can result in completely different symptoms. An 8-month-old girl with glioma of the optic nerve was extremely emaciated, hyperactive and suffered from insomnia. Following partial resection of the tumour and radiological treatment the patient increased rapidly in weight and at the age of 3 years weighed 21 kg.

Another case resembles the syndrome described by Prader and Willi, with psychic and physical retardation, limitation of movement and areflexia. Air encephalography revealed agenesis of the corpus callosum. Glucose tolerance test following administration of hydrocortisone gave the same values as in prediabetes. Skeletal development was retarded, but other signs of diminished thyroid function were absent. A third case was a girl with Laurence-Moon-Biedl's syndrome, where no endocrinological disturbance could be shown.

The mechanism causing obesity in these cases is unknown, but interesting; and it may throw light on the possible significance of the autonomic centres in the development of obesity.

Discussion

F. Karlström. In the pediatric department of Karlstad Communal General Hospital child is now undergoing treatment for diencephalic obesity arising from a leukemia infiltrate in the base of the brain. The patient became ill with leukemia at five years of age and was treated with prednisolone and purinethol. Having had the disease for about one year she developed acute headache, vomiting and paralysis of the eye muscle. Encephalography revealed an expansive process in the central area of the brain. Following treatment with cobalt the eye muscle pareses disappeared and the papilledema became normal, but the patient showed signs of fatigue, dullness and keen hunger. Within three months her weight increased by seven kilograms. Her obesity was more of the so-called Fröhlich- than the Cushing-type. During the last year she has not received steroids and her bone-marrow has been normal. She is now once more being treated with cobalt, directed at the base of the brain.

T. Silwer. *Intussusception in children—difficulty of diagnosis*

Fifty-four cases of invagination in children at the pediatric clinic and the surgical clinic in Kristianstad were reported and it would appear that the symptomatology is not always so unambiguous as stated in many textbooks.

Study of this material revealed a remarkably low incidence of cardinal symptoms—typical recurrent pains occurred in 35 cases, rectal bleeding in 23 cases, vomiting in 32 cases and palpable tumours in 25 cases.

In infants vomiting and rectal bleeding were commoner than typical recurrent pains, but in children above one year old recurrent pains were the predominant symptom.

Most of the children who were admitted directly to the surgical clinic had the characteristic symptomatology with typical recurrent pains as the principal symptom.

Colonic roentgenograms were performed in 50 cases and resulted in diagnosis in 49. The undiagnosed case was one of ileoileal invagination which was not visualised with contrast enema and which resulted in the only death in this material.

Discussion

B. Palmgren. In the Pediatric Hospital at Hälsingborg over the past 10 years there have been 53

patients with roentgenologically-confirmed or surgically-demonstrated intussusception. Of these 37 were boys and 16 girls. The surgical department treated a further 12 cases, of which 9 were boys and 3 girls: these patients were never seen at the Pediatric Hospital.

Recurrent crying or severe pain was the commonest symptom, but here, as in Silwer's study 15% did not have this finding. At this early stage bleeding was observed in only 12 patients, but it was the chief symptom in 7 cases. Fatigue and pallor ar common symptoms, and in 8 patients who were not suffering from pain it was chiefly these symptoms that led to the suspicion of intussusception in 4 cases In two more cases the presenting symptom was bleeding while in the seventh a tumour was palpable. Thirty-six per cent of the cases showed mild symptoms of concurrent infection manifested by either fever or leukocytosis.

A preceding history is important. Of the 65 cases from the Pediatric Hospital and the surgical department 11 were recurrences at widely varying intervals, from 6 days to 20 months.

Roentgenological examinations were performed in 63 cases, and in 45 of these it resulted in a reduction. Eighteen patients, as well as two others who did not have roentgenograms, required surgery Another patient who had symptoms over a long period died following removal of the involved segment. The rest rapidly recovered.

Meeting Nov 30 1966

B Hellström, K. Theorell & U Sallmander *How egularly is prescribed medication taken in cases of epilepsy in children.*

With a material of 158 epileptic children from Karolinska Hospital an interview examination was carried out to ascertain how regularly the prescribed tablet prophylaxis was followed. In 12% of cases it was found that the medication was taken not altogether regularly and 8% were classed as "less satisfactory In some cases failure take the medication was followed by a relapse. No consistent relationship was found between the degree of regularity and certain variables, such as social group duration of the epilepsy frequency of attacks and so on. The ascertained departures from prescription are certainly minimum figures, as no attempt was made otherwise to check regularity The results of this study are reported in more detail elsewhere.

Claes Thorén, Rolf Lundström & Arne Svedmyr *Immunity to German measles in women of child bearing age*

Thanks to virological diagnosis it has become possible during the past four years to carry out intensive research into rubella.

From different parts of the world varying data have been reported concerning immunity to rubella in women of child-bearing age. Immunity can be determined by demonstrating specific antibodies in the blood. The method has hitherto been complicated and time-consuming and therefore unsuitable for large-scale application. Of 200 pregnant women examined in Stockholm and Eskilstuna 88% were immune to rubella. There was no difference between the two cities. The average susceptibility to rubella in pregnant women is thus only 12% which is the same as in North America and Britain but considerably lower than in Japan and Hawaii.

In the Eskilstuna study the position with regard to immunity at various ages was investigated. Immunity increases with age at 15 years old it is about 60% and at 30 years old about 95% Thus it increases more slowly than does immunity to measles—which reflects the differing infectiousness of these two diseases. Evidence of previous rubella infection was in agreement with the presence of antibodies in about 90 of those examined in all ages. Up to about 20 years of age it was relatively unusual that persons who did not believe that they had had rubella nevertheless had become immune. Above the age of 25 however immunity was equally common whether or not there was any history of sickness In view of this it is not surprising that 90% of those women in Stockholm who received gamma globulin prophylaxis against rubella were immune even though they stated that they had not had rubella.

It is probable that adult women can forget the

diseases they had in childhood. A likely explanation of this is that the risks arising from rubella during pregnancy only became generally known in this country following the epidemic of 1951

Some persons can have rubella without any obvious symptoms, which also may explain why they are immune without awareness of previous infection.

Meeting March 11 1967

C. Thorén & J.-A. Hedberg: *Effect of beta-receptor blockade on children at various work-loads*

The beta-receptors produce sympatho-adrenal effects such as dilation of the blood vessels and bronchi, as well as increased heart rate and myocardial contractility. Propranolol (Inderal) is a specific and effective beta-receptor antagonist, of which the hemodynamic effect is characterised by a lower heart-rate and minute volume during work. This result is made possible by increased peripheral utilisation of oxygen and increased RQ.

10 mg Inderal was given orally to 11 healthy children between the ages of 9 and 11 years, inclusive, as well as to some teenagers and cases with orthostatic trouble.

Functional studies with increasing work-loads up to the maximum were carried out by means of an ergometric cycle, with recording of heart rate, blood pressure, respiratory rate, skin temperature, amount of sweating and lactic acid level in the blood. In both age groups the heart rate increased linearly but on a 15–20% lower level following blockade. The drop in heartrate was more pronounced with a heavier work-load. No difference was noted in the respiratory rate, skin temperature or amount of sweating. The ECG generally showed higher *T* waves during and following work with beta blockade, and orthostatic *ST T* changes were normalised.

Following Inderal blockade the lactic values were higher in relation to heart rate, but lower in relation to work-load. In teenagers pyruvate and glucose levels were determined. Pyruvate also was lower but the blood sugar level remained unchanged. Even with the same work load oxygen consumption decreased. The lactate and pyruvate decreases probably also reflect the effect of this substance on peripheral muscular metabolism with improved oxygen utilisation.

The clinical indications for the use of Inderal in pediatrics should be in differential diagnosis of sympatheticotonic ECG-changes, arrhythmias, and some special conditions such as Fallot attacks, cardiomyopathies and pheochromocytoma. In cases of orthostatism during puberty Inderal should prove useful.

L. O. Boréus & B. Sundström. *Intracranial pressure increase of unusual etiology* (Published in *Brit Med J* 1967)

Signs of intracranial pressure increase were observed in a 6-month-old boy following treatment with nalidixan (Negram), for a urinary tract infection. This side-effect of nalidixan has not previously been described. The symptoms consisted of vomiting, bulging of the fontanelle, widening of the sutures and papilloedema. They appeared in three separate phases of nalidixan therapy with normal dosage and led to neurosurgical intervention with exploratory drilling of burr holes. The pressure increase began after a period of 1–3 days therapy with nalidixan and was rapidly reversed after withdrawal of the drug. No permanent ill effects were observed.

C. Thorén. *Treatment of cyanotic attacks with beta-receptor blockade in cases of Fallot's anomaly*

Progressive narrowing of the infundibular stenosis in cases of Fallot's anomaly have been described in several reports. Children with rapidly increasing stenosis run the risk of having "cyanotic spells" which sometimes can be fatal. These attacks result from total occlusion of the infundibulum. In these patients such attacks may be set off for instance by the use of isoprenalin. It has also been shown that during such attacks the stenotic murmur over the pulmonary area decreases considerably.

It was therefore thought of interest to treat 5 children with this anomaly at Kronprinsessan Lovisas Pediatric Hospital by means of the beta receptor blocking drug, propranolol (Inderal). Four of the children responded very well. This was demonstrated partly by heart catheterisation

with cineangiocardiography in one case, where the right-to-left shunt decreased—and in fact a definite left-to-right shunt could be shown—and partly by measurement of the arterial oxygen saturation before and after blockade, and recording of the murmur over the pulmonic area. Moreover two children were treated over a longer period with oral medication. Arterial oxygen saturation increased considerably, the murmur became louder, cyanosis decreased and physical performance improved. Two children stopped having cyanotic spells.

The effect of propranolol in these cases probably depends on inhibition of the spastic narrowing in the infundibulum with increased flow of blood to the lungs.

This drug therefore seems to be the best available for treatment and prevention of cyanotic attacks and unconsciousness in cases of Fallot's anomaly.

Meeting April 23 1967

B. Berglund. *Three patients with acute reticuloendotheliosis treated with Velbe*

Three cases of acute reticuloendotheliosis, where PAD demonstrated changes of Letterer Siwe type, were treated with vinblastine sulphate, Velbe (Lilly).

In one case showing lymphadenopathy and skin lesions, treatment with Velbe was started at the age of 3 months, eight doses of 0.3 mg/kg body weight, each were given intravenously at weekly intervals. The patient improved quickly and during the following two years he has appeared to be completely healthy. PAD from a lymph gland at the age of 18 months demonstrated normal tissue.

The next case developed acute reticuloendotheliosis at the age of 5 months and the diagnosis was verified histologically. First he was treated with steroids and mustard gas, which brought about at temporary improvement. Treatment with Velbe was then instituted. The skin lesions improved rapidly and on completion of the treatment 8 weeks later he was in good health and remained so during the next year of observation. On one occasion a swelling appeared behind his right ear. X-ray showed local bony destruction. After another series of Velbe-Injections, the swelling disappeared. Four months later the X-ray changes had diminished.

The third patient developed acute reticuloendotheliosis at the age of 17 months with typical skin lesions and fever as well as icterus. Laparatomy revealed involvement of the lymph glands in the hilus of the liver as well as in the liver tissue. Initial treatment with steroids and methotrexate produced no noticeable improvement. Somewhat later a series of 8 injections of Velbe was given. During this treatment the skin lesions healed and his general condition improved, although the liver insufficiency remained unchanged. Five months later his condition suddenly deteriorated and he died in liver coma.

In these cases of acute reticuloendotheliosis, Velbe had a beneficial effect on the general condition and on local changes in the skin and lymph nodes. However this material provides an inadequate basis for any judgment as to whether Velbe can influence the prognosis of the disease. The only side effect of Velbe observed in these cases was an arrest of growth during the period of treatment.

H. Haljamäe, S. Hagberg & H. Röckert. *Ion changes in striated musculature in cases of hemorrhagic bleeding in dogs*

For the treatment of serious cases of dehydration we have developed methods for determining the cells' content of sodium and potassium. It was not previously possible to investigate intracellular ion changes in the absence of sufficiently sensitive techniques. Our methods, which are based on roentgen fluorescence and ultraflame photometry can be used at 10^{-1} g. This corresponds to a fragment of dried muscle cells. The methods give absolute values which are related to the mass determined by the roentgen absorption method according to Rosengren. The methods, moreover have been used to study the content of potassium and sodium in the interstitial fluid. The interstitial fluid collects outside the muscle fascia. In the skin an incision is made which is filled with paraf to prevent evaporation. Using an operation microscope, one works down to the fascia, where, with

a quartz pipette one can collect small quantities enveloped in paraffin. This goes through a quartz bowl to form a drop and a measuring pipette calibrated to a volume of 10^{-9} l can be then used. When the absolute quantity of potassium and sodium has been determined the results can be expressed in mEq per litre.

In cases of hemorrhagic shock extracellular potassium increases by 300% and intracellular kalium decreases by 30%.

S. P. Hillström & J. Winberg: *Acrodermatitis enteropathica*

This was a boy 3 years and 8 months old who had had the disease but apparently recovered completely. His sister died of the same disease at the age of about one year. He showed onset of symptoms at the age of 5 months, about one month after weaning. He was then put back on breast feeding and his condition at once improved. Repeated attempts at weaning during the following two years were followed by a deterioration in his condition. Such deteriorations have been previously reported and ascribed to intolerance to cow milk. The patient's condition became worse when the daily supply of breast milk decreased to about 600 g, regardless of whatever foodstuffs (such as cow-milk, cow-milk and cereals, meat and fish, green vegetables, potatoes) were added to his diet to ensure the requisite calorie intake. Our hypothesis is that breast-milk contains some element that to a certain extent neutralises the metabolic disturbance that probably is the basic cause of the disease.

Between 27–33 months of age the patient had his diet supplemented with 40–120 mg vitamin B_6 per day and it became possible, without any deterioration in his condition, to transfer him gradually to a normal diet. Attempts to withdraw vitamin B_6 led, on some occasions, to deterioration, but finally proved successful at the age of 33 months. Enterovioform treatment was gradually reduced and is now down to about one-half tablet twice per day.

As these observations concern only a single patient no general conclusions can be drawn concerning treatment and etiology of the disease but the observations are consistent with the hypothesis that the course of the disease can be modified by the intake of breast milk in large amounts, together with a massive administration of vitamin B_6.

L. Victorin, W. J. Dally, P. Karlberg, T. Olsson & A. Thomson. *Mechanics of breathing and impedance variations*

Newborns with early and late clamping of the cord have been studied with respect to the mechanics of breathing, blood gases and transthoracic impedance variations during the first three days of life.

Up to about 1– hours of age infants with early clamping show a higher compliance, larger respiratory volume and lower P_{CO_2} indicating a relative hyperventilation. In spite of this, P_{O_2} is lower especially between 12– 4 hours. This has been attributed to a shunt through the foramen ovale as the blood samples were taken above the ductus arteriosus via the a. temporalis.

The variations of the impedance curve in general correspond to the respiratory volume changes of the lung as recorded by means of so-called reversed plethysmograph. There are certain dissimilarities, however, probably depending on changes in the amount of blood in the lungs, as the impedance plethysmograph mainly gives a measure of the relationship of air to blood. Preliminary calculations indicate that with early clamping of the cord the lungs contain less blood, but that the displacement of blood with respiration is larger than with late clamping.

This technique seems to provide increased insight into variations in pulmonary circulation in normal as well as pathological conditions. It is also suitable for intensive care observations of newborns with different types of disturbed functional adaptation.

Hans Jörgen Andersen. *Mucoviscidosis treated with N-acetylcysteine*

In vitro experiments have shown that N-acetylcysteine (Mucomyst) can considerably reduce the high viscosity of bronchial secretions in patients with mucoviscidosis (cystic fibrosis of pancreas). Several clinical reports have demonstrated that N-acetylcysteine, given as an aerosol, may help the-

with cineangiocardiography in one case, where the right-to-left shunt decreased—and in fact a definite left-to-right shunt could be shown—and partly by measurement of the arterial oxygen saturation before and after blockade, and recording of the murmur over the pulmonic area. Moreover two children were treated over a longer period with oral medication. Arterial oxygen saturation increased considerably the murmur became louder cyanosis decreased and physical performance improved. Two children stopped having cyanotic spells.

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earlier study that was identical, apart from the fact that most of the mothers were delivered in the 36th–37th week, the incidence in the Borås material was significantly lower

A.-L. Bergstrom & E. B Möller *Screening method for early recognition of hyperbilirubinemia in newborns*

A screening method described by Streicher in 1962 and based on determination of the bilirubin content in umbilical cord blood has been evaluated and modified in an investigation involving nearly 5000 infants born at Borås maternity clinic during a period of two years. The material included premature infants with a birth weight of more than 2000 g and only a few cases of Rh-immunisation that were not discovered at the usual pregnancy examinations. Bilirubin was determined by the usual hospital technique (Jendrassik–Gróf, modified by Michaëlsson) within 4–24 hours after delivery

A risk of hyperbilirubinemia of varying etiology developing and requiring exchange transfusion was found to arise when the umbilical cord bilirubin level reached 3.2 mg/100 ml, or more. The group subject to this risk comprised only 3.5 % of the cases, so that after screening there are only a small number of children requiring examination by a special clinic. This comparatively simple screening method should prove particularly useful in delivery units which do not enjoy the services of a pediatric consultant.

Meeting Oct. 21 1967

G Meeuwisse, Y Naversten & R. Nilsén: *Renography and kidney scintigraphy in children*

Renography is a dynamic kidney function test performed by means of a tracer labelled with a radionuclide. ^{131}I-Hippuran is widely used in adult patients. However as regards radiation characteristics in small children ^{125}I is more suitable than ^{131}I. The authors therefore have used ^{125}I-Hippuran.

The instruments used in this study were two small matched detectors, each collimated, fitted with a NaI(Tl) crystal and a photomultiplier and attached to a rate meter and a strip chart recorder The renography was performed during 20–30 minutes following i.v injection of 50 c of the tracer Immediately afterwards, over a period of 10–15 minutes, the lumbosacral region was completely scanned with an organ scintigraph.

The renograms were interpreted using the simple criteria of Nosslin & Denneberg. Only two infants with normal function were studied. Normal values for children do not exist, but there are probably no large deviations from adult standards

Some typical cases were demonstrated, including one infant with normal kidney function, one with bilateral kidney hypoplasia, one with unilaterally decreased function and one older child with aplasia of one kidney and hydronephrosis of the other side. The kidney function studies with ^{125}I-Hippuran, as performed by the authors, were found to form a valuable complement to other function tests and to X-ray examinations of the urinary tract The renogram discloses—and to some degree quantitates—differences in the capacity of the kidneys, and it may reveal stasis in the urinary tract of infants in whom i pyelography has been unsuccessful. In several infants the scintigram localized kidneys that could not be detected by i.v pyelography

Bengt Kjellman. *Regional lung function studied with* ^{133}*X in children with pneumonia.* (Published in *Acta Paediat Scand*, 57 467 1967)

Meeting Dec. 1 1967

Leif Hambraeus, Irène Sjögren & Olof Ulwán. *The aminoacid pattern of cerebrospinal fluid in infantile hydrocephalus*

Analyses of the amino acid pattern in 23 specimens of cerebrospinal fluid from 19 children (9 boys and 10 girls) with infantile hydrocephalus have

been performed by means of thin-layer chromatography The specimens were obtained by ventricular puncture (16 specimens) or by lumbar puncture (7 specimens)

Results indicated that the specimens were separable into three groups. The largest group comprising 17 specimens from 15 patients, showed an amino acid pattern of the same type as earlier described in studies by other authors, the pattern being characterized by the glutamine concentration. In the second group comprising 4 specimens from 3 patients, a slightly increased amino acid pattern of the generalized type was recognized In all cases there was involvement of the blood-CSF barrier (meningitis due to a listeria infection, subdural hematoma and spina bifida cystica, respectively) The third group comprised [illegible] specimens from a patient with hydroanencephalia. In this case a very markedly increased amino acid pattern was found Quantitative amino acid analyses by means of an automatic amino acid analyzer revealed that the concentration of all except two of the naturally-occurring amino acids had increased from three to seven times. The concentration of glutamine and glutamic acid was, however significantly decreased, being 3 μmoles per 100 ml cerebrospinal fluid.

J Winberg, P Unger & R. Zetterström. *Fatal granulomatosis*

Four boys are described showing the typical features of multiple granulomas of internal organs, from which staphylococci or gram-negative organisms occasionally were isolated. Two of the patients had intestinal infiltrations with marked abdominal symptoms. Malabsorption of vitamin B_1 with beri-beri heart in one of them and severe hypoproteinaemia in the other were probable secondary effects of these infiltrations.

Different aspects of the immune defense mechanisms were investigated. The results can be summarized as follows:

Humorally mediated defence

Non-specific

Bactericidal activity Normal
Viricidal activity Normal
Serum complement: Normal

Specific

Immune globulins: Normal
Polio vaccination. Normal response
Isoagglutinins. Normal titers

Cellularly mediated defence

Non-specific

Leukocytes. Normal number (often eosinophilia)

Specific

Tuberculin reaction positive

Thus the aspects of immune defence which commonly attract interest were normal. There were no side-effects following BCG or smallpox vaccination The course of viral infections was uneventful.

Recently Good *et al.* have shown that the immune defect in this disease lies in an inability of the granulocytes to destroy phagocytosed Staphylococci and certain Gram-negative organisms.

Our four patients were noteworthy in several respects

1 Three of them attained a relatively high age 15 $/_2$ years (dead) 17 years and 13 $^1/_2$ years.

2. One patient, who had been in relatively good condition was given prednisolone because of severe muscular pains. The symptomatic effect was good but he died 3 months later with widespread granulomas.

3 Tetracycline was shown to be of special value in treating the infections.

4 The mother of one of the patients had a panophthalmia caused by salmonella, and subsequently for 3 years she had fistulating abscesses on one hand from which *E coli* was isolated. This is probably a clinical manifestation of the carrier state

G Wessner K. Lincoln & J Winberg *Neonatal E. coli meningitis*

Nineteen patients with neonatal purulent meningitis not associated with myelomeningocele were observed in the Gothenburg area 1959–1967 five of these during the last year In addition two were referred from other hospitals. The table show the prognosis which—with reservation for short observation time—seems to have improved the last year

	Prognosis in *E. coli* meningitis	
	1959–66	1966–67
Total no. of patients	14	7
Healthy	4	6
Dead	10	1

An analysis of different factors suggest introduction of kanamycin in the treatment to have been the decisive one for the change of prognosis.

Adequate duration of treatment cannot be predicted as safely as in the common types of purulent meningitis. Neg. culture, decreasing values for cells and protein in the CSF were not reliable in evaluation of the effect of therapy. Our study suggests that analysis of the CSF/blood sugar quotient at the end of therapy and at repeated examination the days following omission of therapy may provide a better method for evaluation.

Pathogenetic factors were shortly analysed. There was no obvious overrepresentation of obstetric complications usually judged to predispose to infection. The type of feeding before appearance of the first symptoms of infection was analysed in 34 patients with proved or highly probably *E. coli* sepsis appearing before the 10th day of life. Consumption of the own mothers breast milk was in this infected group significantly less than in 70 controls.

R. Lagercrantz

PROCEEDINGS OF PEDIATRIC SOCIETIES

FINNISH PEDIATRIC SOCIETY

Meeting Feb 17 1968

Odile Schweisguth (Institute Gustave Roussy France): *Treatment of solid malignant tumors in children*

A correct diagnosis is the first condition for successful treatment of malignant tumors. The histologic examination of the tumor tissue may be of great value for the clinician, but also the pathologic-anatomic diagnosis may be incorrect, especially if the specimen is taken from a necrotic part of the tumor. When assessing the spread of a malignant process, urography arteriography and lymphography have turned out to be of great value.

The treatment of patients with malignant diseases consists of a combination of radiotherapy chemotherapy and surgery. The best results are achieved when these patients are under the supervision of an experienced team consisting of a radiologist, a chemotherapist and a surgeon.

In children a two years follow-up corresponds in most instances to a five years follow-up in adults. However the absence of symptoms after two years does not prove final healing. For instance in thyroid carcinoma a relaps may occur even *after 15 years*.

The combination of different forms of treatment was illustrated by a series of cases.

In Wilm's tumor the author uses routinely preoperative radiation with ?000 r during two weeks. Thereby the size of the tumor is reduced and it is easier to ligate the renal vein without squeezing the tumor thus reducing the risk of metastatic spread of tumor cells. The postoperative therapy depends on whether the surgeon has observed adhesions, metastatic lymph nodes or infiltration of the tumor into the inferior vena cava. If no such signs are observed the patient does not receive postoperative radiation. When infiltrations are observed the postoperative radiation is facilitated if these infiltrations are marked with metal clips during operation. Actinomycin D is never used preoperatively since it causes thrombopenia, but postoperatively Actinomycin D clearly improves the results. Cases with solitary lung metastases have been cured in 20–25 per cent.

In neuroblastoma good results were often achieved with the combination of Sendoxan and Vincristine. If the tumor is localized, radiation is also used. *The prognosis depends more on the age* of the patient than on the way of treatment, and is best in children under one year of age. The prognosis is better in intra-thoracic neuroblastoma than in intra-abdominal neuroblastoma, probably because the cells are more differentiated in the former cases. Radiation should be avoided *in infants below one year of age because of the* severe side effects on the growing organism.

In ovarian teratoma trippeltherapy with Sendoxan, Metotrexate and Actinomycin D may be of great value.

Treatment of malignant tumors is not a hopeless task. The results cannot be expected to be *good in every case, but it is good for the parents* to know that everything has been done for their sick child. The child also needs help during the last stage of the disease. It is the duty of the physician to help the family through this difficult time.

E. I. Wallgren

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BOOK REVIEWS

F Gronlich. *Die Rezeptorfunktion de Erythrozyten.* 158 pp Illus. Bibliotheca Haematologica, Fasc. 25 S. Karger AG, Basel and New York 1966. Sw Fr. 38.

The purpose of this book is to present survey of and experimental studies on the interaction between the red blood cell surface and various substances. The survey part (82 pages) is mainly devoted to reactions between red blood cells with substances occurring within the species (iso- and auto-antibodies, plasmaproteins) and species-foreign substances. The experimental part (51 pages) describes in detail coating of red blood cells with various substances (mainly serum protein fractions) and the "natural" plasma protein coating of erythrocytes from essentially quantitative point of view The last part of the book is entitled methods and material and states in 18 pages the methods used in the author' studies. The bibliography lists 429 references. The book may be of interest for research orkers studying for example the relation between erythrocyte surface and the binding of various substances to this surface.

Jan Hirschfeld

John Money: *The Disabled Reader Education of the Dyslexic Child*, 421 pp illus. The Johns Hopkins Press, Baltimore 1966. $8.50

The editor and thirteen contributors discuss reading disability and the alternative methods that may ameliorate it. Some of them maintain that reproduction (spelling) should be delayed until recognition (reading) has been established, others start with kinesthetic and motor type of spelling and still others combine it.

The value to Scandinavian readers is limited in the fact that the emphasis of this book is placed on the English language and that the problems of learning English are different from those of learning Scandinavian languages.

Britta Sandström

Heinz Spiess. *Schutzimpfungen*, 370 pp illus. Georg Thieme Verlag, Stuttgart 1966 DM 45.

The need of complete and comprehensive text-book about vaccines and their use in preventive medicine is very obvious, so the second revised edition of Professor Spiess *Schutzimpfungen* is received with great satisfaction and expectation.

The book is extremely detailed and complete and has many good illustrations, but is easy to state that great differences exist between different countries not only concerning the intervals between the vaccinations but also between the more basic principles.

As long as there does not exist any sufficient international standards for different vaccines statement of dosage ill be of little alue.

Anybody with fundamental knowledge in this field who is looking for detailed information will find it orth while reading this work which is also provided with numerous literature references.

Agneta Philipson

H. Opitz & F Schmid (eds.): *Handbuch der Kinderheilkunde* Vol. IV Stoffwechsel, Ernährung, Verdauung, 1 43 pp. Springer Verlag, Berlin, Heidelberg, New York 1965.

The metabolism, the nutrition, and the digestion have been discussed in three approximately equal parts. The section on metabolism deals with the physiological and pathological metabolism of amino acids, proteins, carbohydrates, lipids, water minerals, and vitamins. The section on nutrition contains chapters on general and special nutrition problems at different ges, disturbances in nutrition and dietetics. The section on digestion contains first chapter on symptomatology and then the different parts of the gastrointestinal tract and related tissues are discussed separately (mouth, oesophagus, stomach, small intestine, large intestine, peritoneum, omentum, diaphragm, liver and pancreas). In all 59 authors have contributed. Many of them are well-known specialists on the subjects treated.

The book appears to be very carefully edited, and each chapter has number of sub-headings (definition of syndrome, clinical picture, historical, genetical, pathogenesis, laboratory findings, diagnosis, differential diagnosis, therapy prognosis etc.) hich makes it easy to find the information one is looking for There are numerous literature references covering hat was published in 1963 and earlier

A minor point has been noted. In the section on metabolism the coenzymes NAD and NADP are named by their old names DPN and TPN Since the new nomenclature is now generally accepted there is no reason to stick to the old one

The illustrations are numerous and ell selected. The book contains large bulk of information, and it ill surely become valuable handbook for specialists in pediatrics and related fields

Arne Dahlqvist

Marcel Fèvre *Chirurgie I fantile et Orthopédie* 2 vols 1681 pp illus Editions Médicales Flammarion, Paris 1967 Sw Cr 480

This is the first modern text-book in French on Pediatric Surgery ritten by the Head of the Department of Pediatric Surgery of the Hôpital des Enfants Malades in Paris The entire field of pediatric surgery is covered, xcept cardiovascular anomalies and neurosurgery. Orthopedic surgery ntl part of pediatric surgery in some countries th entire second volume (680 pages) is devoted

to orthopedic conditions. The text is up to date, clear and systematic. There are many good illustrations. The book is rather heavy—the table of contents alone comprised 30 pages, which is significant of the thoroughness of this work.

Readers who prefer or are equally familiar with English will find the American *Pediatric Surgery* (Year Book Publ., 1962; second edition is due in July 1968) more palatable

Th. Ehrenpreis

Erich Zapp: *Urologie des Kindesalters*, 320 pp., Illus. Ferdinand Enke Verlag, Stuttgart 1967 DM 65

In spite of good intentions the presentation is unfortunately pedantic, inequable, partly outmoded or inexact, sometimes uncritical or too certain. It gives the impression that the uthor experience in urologic diagnostics and surgical therapy presumably still is rather limited. The typography is excellent.

N O Ericsson

John D Keith, Richard D Rowe & Peter Vlad *Heart Diseases in Infancy and Childhood* 2nd ed., 1239 pp. The Macmillan Co., New York, N Y 1967 300s.

Almost ten years after the first edition of this well known textbook in pediatric cardiology it is now time for second revised edition. The reviewer has thoroughly "tested" and used this last edition as reference source in *the daily routine activities of* pediatric cardiovascular laboratory for more than half a year Although some recent contributions, such as the ingenious method of Rashkind for septotomy in complete transposition f the great arteries, are not included in the issue, the revision of the first edition has brought this volume up to date with respect to pertinent therapeutic trials and statistics. Revision has extended to complete re-writing on such major topics as the septal defects, transposition of the great arteries and the tetralogy of Fallot.

The clear outline makes the abundant supply of information easily found and read. As a minor criticism some of the accompanying radiograms do not match the high standard of the book as whole. In the reviewer's opinion it is unfortunate that the three-diameterformula for the calculation of heart volume has not yet managed to replace the "cardio-thoracic index on the other side of the Atlantic.

The authors have accomplished the task of writing book which has become classic in pediatric cardiology since the appearance of the first edition less than 10 years ago. The second edition will undoubtedly further strengthen this position.

Göran Wallgren

J L Melnick (ed): *Progress in Medical Virology* vol. 9 496 pp. (+53 figs.). S Karger AG Basel, New York 1967 U.S. $21 10- Sw kr 123 70.

As with previous volumes, the present issue of *Progress in Medical Virology* is of great interest to everyone orking in the field of infectious diseases. The book is a successful combination of basic virologic data and clinical aspects of viral infections and the editor has, as usual, chosen topics of great current interest. This volume contains several chapters of principal interest to the paediatrician. This is particularly true of the review by Eichenwald, McCracken & Kindberg of recent investigations of virus infections of the newborn. The chapter as a review is *fairly comprehensive and though it lacks* certain references to recent European studies, is still very informative. Another topic of paediatric interest is the excellent comparative appraisal of hemorrhagic fevers of Southeast Asia and South America by Jonson, Halstead & Cohen. The evidence to date strongly suggests new concept of the pathogenesis of the disease of Southeast Asia hypersensitivity response occasioned by second or third dengue virus infection. If this can be substantiated it will also be of great importance with respect to insight into hypersensitivity diseases in man. There are also other subjects rather closely related to paediatrics such as the presentation by Vonka, Janda, Simon, Adam & Starek of new and probably more suitable type 3 attenuated poliovirus for possible use in oral polio irus vaccine.

Many of the subjects are of great general interest, i.e. Lockart distinguished discussion of the rapidly developing field of interferons, Plummer informative summary of new data in the herpes virus group and Bell' excellent review of the numerous efforts to establish aetiologic association of variety of viruses with the malignant African lymphoma, Burkitt tumor Warwick gives a *comprehensive summary of the background of the cat* scratch syndrome. So far the few reports of isolation of specific etiologic agent remain unconfirmed. Boiron, Lévy & Périés survey the *in itro* investigations of the viruses of murine leukemia, the only leukemia of mammals in which a viral etiology has been firmly established.

Epidemic encephalitis caused by arboviruses represent threats to human life and health around the globe not only in countries where the disease may be endemic but also in countries having no past experience of the iral agent. Phillips & Melnick review the large outbreak of St Louis-encephalitis in 1964 in Houston, T xas, and Ross describes the severe epidemic of Venezuelan equine encephalitis in 1962–63 in Venezuela

Some parts of the book deal with more basic virologic topics. Raben & Jenson summarize the information rapidly accumulating from electron microscopic studies of *in vi* viral infections and finally Wildy Ginsberg, Brandes & Maurin contribute chapter on virus classification

This volume may be recommended to irologist as well as clinicians. The paediatrician in particular will find useful knowledge not only in the articles closely related to paediatrics but also in the reviews of subject ith a more general outlook.

Gun Carlström

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GLUCOSE-GALACTOSE MALABSORPTION

A Study with Biopsy of the Small Intestinal Mucosa

G W Meeuwisse and A. Dahlqvist

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Glucose-galactose malabsorption is an inherited, congenital disease in which the intestinal absorption of glucose and galactose proceeds very slowly (1 19 21–25 27 33) The administration of these sugars, either as monosaccharides or as digestible oligo- or polysaccharides, to patients with glucose-galactose malabsorption causes severe diarrhoea. The absorption of fructose is normal and—as has been shown by intestinal intubation studies (21)—in patients with glucose-galactose malabsorption the absorption of fructose proceeds more rapidly than the absorption of glucose. Consequently it has been suggested, that the patients have a specific defect in the active glucose transport system present in the normal small intestinal mucosa (21 33). In order to obtain more evidence for this theory the absorption *in vitro* was measured by incubating intestinal biopsy specimens from patients suffering from glucose-galactose malabsorption with D-glucose and the amino acid L-alanine We have also studied some related properties. the inhibition of intestinal ATP-ase activity by ouabain, the sodium activation of the jejunal invertase, sodium transport *in vivo* quantitative disaccharidase activities and the histological structure of the mucosa. A preliminary report has been published (29).

MATERIAL

One child and one adult with glucose-galactose malabsorption were studied Some investigations were also performed on intestinal biopsy specimens from the parents of the younger patient.

Case 1[1]

Glucose-galactose malabsorption. Girl aged 16 months with watery diarrhoea from birth. The diarrhoea could be stopped only by feeding a glucose-galactose-free formula diet, containing fructose as the only carbohydrate. Diagnosis was confirmed by sugar loading tests and an intubation study which showed fructose to be absorbed faster than glucose. She also had mild renal glucosuria, as has been found in most patients with the disease.

Case 2

Glucose-galactose malabsorption. Woman aged 38, member of a family with several cases of glucose-galactose malabsorption (30). Diagnosis was confirmed in the same way as in case 1 (22).

Control subjects. As control material was generally used mucosal specimens from children undergoing intestinal biopsy for various clinical reasons, but in whom no structural abnormality and no disaccharidase deficiency was found. However as control material for the study of the Na activation of intestinal invertase, mucosa from a surgically obtained piece of jejunum from an adult was used, and as a control for the incubation study and the *in vitro* inhibition of glucose transport by phlorizin, an apparently healthy adult was used.

METHODS

Intestinal biopsy

Biopsy specimens, weighing about 10 mg, were taken near the ligament of Treitz with a Lehmann hydraulic biopsy instrument (20) or with a similar hydraulic apparatus especially constructed for use in infants (28). The biopsies were flushed out from the capsule with isotonic sodium phosphate buffer pH 7.4.

Histology Biopsy samples were immediately fixed with buffered 10% formaldehyde for light microscopy and with 6.5% glutaraldehyde in phosphate buffered saline for electron microscopy

Incubation with ^{14}C*-labelled compounds*

Incubation with ^{14}C*-labelled D-glucose* Fresh biopsy specimens were preincubated for 20 minutes in Krebs-Hen-

[1] The child was kindly referred by Dr K. Kayser (County Hospital of Eskilstuna), who will describe the clinical history of this case in detail (17).

Uniformly labelled ^{14}C-compounds were used

teleit buffer (15) at 37°C continuously bubbled with 95% O_2 and 5% CO_2 through a fine cannula. The biopsies were then transferred to test tubes containing 1 ml of the same buffer with added glucose (2.5–20 mM), labelled with [14]C to give about 300,000 counts per min per ml. In some experiments phlorizin (0.1 mM) was present in the solution. Continuous mixing was effected by bubbling with the O_2-CO_2-mixture as during preincubation. The incubation lasted 10–60 min at 37°C. Afterwards the biopsies were gently blotted, weighed, then immediately frozen and stored at −20°C for one or two days. Before analysis the biopsies were homogenized with ml distilled water in motor driven glass pestle homogenizer chilled with ice. Radioactivity was measured in Packard Tri-Carb-scintillation spectrometer. Each 0.5 ml sample was mixed with 10 ml scintillation liquid, a slightly modified Bray's solution of the following composition: 60 g naphthalene, 4 g PPO 0.2 g dimethyl-POPOP 100 ml metanol, 20 ml ethylene glycol and dioxane to 1000 ml. The amount of [14]C-labelled glucose absorbed by the tissue was expressed as per cent filling of the mucosa. This was calculated in the following way: per cent filling = tissue conc. 100/medium conc. 0.8 The figure 0.8 represents the water content, assumed to be 0.8 times the wet weight of the biopsy (3 7). The glucose content of the homogenized intestinal mucosa was also measured enzymatically with TRIS glucose oxidase reagent (10) after deproteinization of the samples with barium hydroxide and zinc sulfate (36).

Incubation with [14]C-labelled L-alanine. The incubation with L-alanine was performed in the same way as described above for glucose. The incubation medium contained 1 mM L-alanine. Radioactivity was determined and calculated as per cent filling of the mucosa in the same way as for glucose.

Enzyme activities

Disaccharidase act ity assay 10–1 mg mucosa was homogenized with 0.2 ml of distilled water in the motor driven, ice-chilled glass pestle homogenizer. Disaccharidase activities were measured by the method of Messer & Dahlqvist (31). Protein was assayed using the method of Lowry et al (26) and the enzyme activities were e pressed as units per gram of protein. One enzyme unit is the activity hydrolyzing 1 μmole of substrate per minute at 37°C.

Sodium act ation f invertase Mucosal homogenates were dialyzed against large volume of distilled water for 4–48 hours at +4°C in order to remove sodium ions. Invertase activity was measured as described by Dahlqvist (10) but the sodium maleate buffe was replaced by lithium maleate buffer of the same pH. Vary ing amounts of NaCl were added to study the activation of the enzyme by sodium ions (34).

Alkaline phosphatase act ity Alkaline phosphatase activity was assayed by modification of the method of Bessey et al. (') using p-nitrophenyl-phosphate as the substrate, in the presence of magnesium, zinc and cobalt ions as activators (4). This method has previously been used for the assay of alkaline phosphatase activity in peroral biopsy specimens of the small intestinal mucosa (13). One unit of alkaline phosphatase is the activity hydrolyzing 1 μmole of p-nitrophenyl phosphate per minute at 37°C. The alkaline phosphatase activity is pressed as units per gram of protein.

ATP-ase activity The ATP-ase activity was measured with the method of Taylor (37) with and without ouabain (0.2 mM). One unit of ATP-ase is the activity liberating 1 μmole of phosphate per minute at 37°C. The ouabain-sensitive fraction of the ATP-ase is expressed as per cent of total ATP-ase activity

Perfusion studies in vivo

After an overnight fast a double-lumen plastic tube was passed into the small intestine under fluoroscopic control. One lumen of the tube discharged in the middle part of the duodenum. The other lumen reached 25 cm more distally At a flow rate of 7 ml per minute an isotonic solution, containing [22]Na-labelled NaCl to give about 5500 counts per min per ml, 12 mM mannitol or glucose and 0.2% PEG (polyethylene glycol, M. W 4000) was infused continuously through the upper hole with the patient lying in the left lateral position. Samples ere taken through the lower hole ith continuous suction of about 30 cm water First mannitol was perfused for 60 minutes, followed by perfusion with glucose for 40 minutes and then mannitol was perfused again for 40 minutes The patient was finally perfused with mannitol and NaCl without the [22]N label in order to measure the recirculation of absorbed [22]Na. Samples were collected for periods of bet een 2 and 10 minutes. On analyzing the samples it was found that after a change of infusion solution a steady state was obtained within 20 minutes. Only the last 3–5 samples in each period, when at least 20 minutes had passed after a change of solution, were therefore used for calculation of the absorption rates. Total sodium concentration was measured with an Eppendorff flame photometer [22]N was measured in a Packard Tri-Carb-scintillation spectrometer ith the same scintillation liquid as for [14]C or in a Packard Autogamma spectrometer with a crystal detector The net absorption of sodium as calculated from the flame photometric assay the bsorption of administered sodium was calculated from the radioactivity measurements. The following determinations were performed after deproteinization of the samples with barium hydroxide and zinc sulfate (36): Glucose in the separated samples as assayed with TRIS-glucose-oxidase reagent (10). Assay of mannitol was performed by the method of Corcoran & Page (5). The PEG content of the samples as deter mined by the method of Hydén (15). Absorption rates —μmoles (mequiv) min—were calculated by the formula $V(C_i - C_r(M \quad M_r))$ in which V is the infused volume in ml per minute, C and C ar the concentrations in mM (mN) of the studied compound in infusion solution and recovered intestinal juice, respectively and M and M the corresponding concentrations of the unabsorbable marker (PEG).

RESULTS

Histology The morphology of the jejunal mucosa from both patients as seen in the light microscope was normal. In the electron microscope the mu-

Table 1. *Per cent filling of mucosa after incubation with ¹⁴C-labeled glucose of varying concentration for 10 minutes*

Samples from the child with glucose-galactose malabsorption, from the patient's parents and from a control subject (infant). Values without brackets were calculated from radioactivity measurements. Values within brackets were found by biochemical glucose determination

Glucose concentration (mM)	Per cent filling of mucosa Patient	Father	Mother	Control
2.5	100 (50)	145 (64)	—	315 (120)
5.0	60 (5)	220 (95)	360 (220)	40 (100)
10	66 (8)	155 (118)	—	245 (125)
20	66 (21)	100 (45)	—	125 (75)

Table 3. *Per cent filling of the mucosa (measured by radioactivity) after incubation with 1 mM ¹⁴C-labeled L-alanine*

Samples from the child with glucose-galactose malabsorption and three control subjects

Time of incubation (min)	Per cent filling of the mucosa Patient	Control subject 1	Control subject 2	Control subject 3
5	—	193	152	—
10	300	436	252	—
20	—	320	434	548
40	—	—	—	625
60	510	—	—	—

cosal cells from the child showed a somewhat sparse endoplasmic reticulum, but otherwise the picture was quite normal. Electron microscopy of the biopsy specimen from the adult patient was not performed.

Glucose absorption in vitro. Small-intestinal mucosa from the patients with glucose-galactose malabsorption did not concentrate radioactivity above the medium concentration when incubated with ¹⁴C-labeled glucose in oxygenated Krebs-Henseleit buffer for 10–60 minutes (Table 1–2). Both parents of case 1 concentrated ¹⁴C to above the concentration of the medium within 10 minutes, although the accumulation effected by the mucosa of the father was rather small. The father's mucosa was also incubated for longer periods with 5.0 mM ¹⁴C-labeled glucose. After 30 and 60 minutes a filling of 194 and 276 per cent, respectively was obtained. Chemical glucose determination gave lower per cents of filling than the radioactivity measurements. The difference seems to be caused by metabolism of glucose after entry into the mucosa. Both parents concentrated glucose to above the concentration of the medium also when the results of the chemical assay were used for the calculation of per cent filling (Table 1 figures within brackets.) The chemically determined glucose filling of the biopsy specimens from the father incubated in 5 mM glucose for 30 and 60 minutes was 114 and 146 per cent, respectively. The presence of phloridzin did not further decrease the glucose uptake by the mucosa from a patient with glucose-galactose malabsorption.

Table 2. *Per cent filling of the mucosa after incubation with 5.0 mM ¹⁴C-labelled glucose in the presence and absence of phloridzin* (0.1 mM)

Mucosa from the adult patient with glucose-galactose malabsorption and from an adult control subject. Values calculated from radioactivity measurements. Values within brackets were found by biochemical glucose determination

Time of incubation (min)	Per cent filling of mucosa: Patient, Without phloridzin	Patient, With phloridzin	Control subject, Without phloridzin	Control subject, With phloridzin
20	58	36	625 (39)	290 (120)
60	78	81	1285 (832)	415 (75)

Alanine absorption in vitro. The L-alanine uptake by the mucosa from the child with glucose-galactose malabsorption was similar to that from three control subjects (Table 3). In all cases ¹⁴C was accumulated in the tissue against a concentration gradient.

Disaccharidase and alkaline phosphatase activities. Disaccharidase and alkaline phosphatase activities of the homogenized mucosa from the adult patient are listed in Table 4. All values are normal. From the child too few biopsies were obtained to permit these assays. The sodium activation of invertase in the mucosa from the adult patient in comparison with a control subject is seen in Table 5. The invertase from the patient was activated in the same way as the invertase from the control subject.

ATP-ase activity. The total ATP-ase activity in the mucosa from the child with glucose-galactose malabsorption was 53 units per g protein. In the

Table 4 *Disaccharidase and alkaline phosphatase activities of homogenized mucosa from the adult patient with glucose-galactose malabsorption*

	Units per gram of protein		
		Normal values after Dunphy *et al.* [13]	
Enzyme	Patient	Mean (*n* = 22)	Range
Maltase	490	160[a]	66–252
Isomaltase	192	97	25–183
Invertase	164	87	26–138
Trehalase	72	—	—
Lactase	95	43	9–98
Alkaline phosphatase	231	802	201–2540

[a] Recalculated for difference in method (31).

Table 5 *Na^+-activation of the intestinal invertase from the adult patient with glucose-galactose malabsorption and an adult control subject*

NaCl concentration (moles per liter)	µg of glucose liberated from sucrose during 60 min	
	Patient	Control subject
0.000	13.0	17 0
0.025	23.5	28.0
0.050	24.5	28 5
0.100	24.5	27 5

presence of 0.2 m*M* ouabain the ATP-ase activity was 14% lower In four control subjects the ATP-ase activity was 35–65 units per g protein and 12–39% of the total activity was inhibited by ouabain.

Absorption studies in vivo. The absorption rates of mannitol, glucose and sodium ions during perfusion of 25 cm upper intestine are seen in Table 6–7 As expected, the glucose absorption in the patient was much lower than in the control subject. When a glucose-free solution was infused (mannitol instead of glucose), glucose was ex into the lumen of the gut at a rate of 3–4 µmoles per minute per 25 cm intestine—no such excretion was seen in the control subject. It should be noted, that the absorption of glucose in the patient was considerably below that of mannitol, even if correction is made for excretion of glucose. Wide variation in net sodium absorption was observed in the patient as well as in the control subject (Tables 6–7) apparently depending on fluctuations in the sodium secretion rate, because the absorption of radioactive sodium was rather constant in each subject. Only small amounts of labelled sodium were secreted at the end of the experiment on the patient (Table 6). Consequently recirculation as a source of error can be neglected. The patient seemed to absorb the isotope about twice as fast as the control. Also mannitol was better absorbed by the piece of gut perfused in the patient. The rates of ^{22}Na-absorption from a perfusate containing glucose did not differ significantly from the rates observed when glucose was replaced by mannitol

DISCUSSION

The results of our experiments have shown that intestinal mucosa from the patients with glucose-galactose malabsorption lacks the ability to ac-

Table 6. *Absorption rates of mannitol, glucose and sodium ions during intestinal perfusion with isotonic solutions in the adult patient with glucose-galactose malabsorption*

Negative values represent net secretion of sodium ions

	Absorption, µmoles (µequiv)/min/25 cm intestine, mean and range (within brackets)			
			Sodium ions	
Compounds infused	Mannitol	Glucose	Chemical assay	Radioact. assay
Mannitol, 12 m*M* and ^{22}N	20.5 (19.2 22.4)		187 (154–241)	725 (650–791)
Glucose, 12 m*M* and ^{22}N		5.3 (3.9–5.5)	164 (120–213)	686 (684 688)
Mannitol, 1 m*M* and ^{22}Na	24 9 (17 1 29.2)		55 (−41 155)	650 (632–678)
Mannitol, 12 m*M* and cold Na	15 7 (15 1 16.8)		151 (107 194)	4 (23– 25)

Table 7 *Absorption rates of mannitol, glucose and sodium ions during intestinal perfusion with isotonic solutions in an adult control subject*

Compounds infused	Absorption, μmoles (μequiv)/min/25 cm intestine, mean and range (within brackets)		Sodium ions	
	Mannitol	Glucose	Chemical assay	Radioact. assay
Mannitol, 12 mM and ^{22}Na	4.3 (2.0–6.6)		−39 (−92–+29)	304 (273–331)
Glucose, 12 mM and ^{22}Na		47.0 (44.1–53.3)	−47 (−107–+24)	339 (304–377)
Mannitol, 12 mM and ^{22}Na	3.6 (0–7.6)		−249 (−282–−162)	326 (277–382)

cumulate glucose. This agrees with the results of Schneider *et al.* (33) who with an autoradiographic technique did not find any evidence of galactose accumulation in incubated mucosa from a patient with glucose-galactose malabsorption. Recently also Eggermont & Loeb (14) have reported lack of accumulation of D-glucose by *in vitro* incubated intestinal mucosa from a patient with glucose-galactose malabsorption. The method used in their study, performed simultaneously and independently of ours, was essentially the same. Accumulation of glucose by the mucosa of the parents of one patient agrees with the previously reported recessive mode of inheritance of the disease. The normal value in the mother (much to our regret from only one biopsy) speaks against the suspicion that the heterozygotes might have a reduced rate of glucose absorption. The rather slow accumulation by the mucosa from the father is therefore difficult to explain. Unfortunately no further investigations could be performed on him.

The normal histology of the mucosa from our patients and the normal disaccharidase and alkaline phosphatase activities support the theory that the disease is confined to the specific glucose and galactose transporting mechanism and that the decreased glucose absorption is not secondary to mucosal damage. The finding of a somewhat sparse endoplasmic reticulum by electron microscopy is non-specific and has probably no diagnostic significance. The normal L-alanine absorption gives contributary evidence for the specificity of the defect in glucose-galactose malabsorption, as does the finding of Eggermont & Loeb (14) that L-leucine was normally accumulated by the mucosa from a patient with glucose-galactose malabsorption. The absorption of glucose and galactose in normal intestine is assumed to be effected by a specific carrier moving the hexose across the cell membrane. Crane and associates postulate that this step is not energy-dependent (6). Glucose and galactose (and some other sugars with appropriate structure but without nutritional significance) share the same carrier. The affinity of the carrier for the sugar is affected by the sodium ion concentration in such a way that it will tend to remove sugar from the lumen, and accumulate it inside the cells (6). It is the maintenance of the normal intra- and extra-cellular sodium concentration difference and not the sugar transport per se which is energy dependent. The Na-K-activated, ouabain-inhibited ATP-ase ("pump ATP-ase"), which can be measured in mucosal homogenates, is assumed to reflect the activity of the sodium pump which maintains the concentration difference (35–37).

If we accept this theory, the inability of the mucosa from the patients to accumulate glucose may be caused by a fault either in the carrier mechanism or in the energy dependent sodium pump. The evidently normal pump ATP-ase activity and the normal accumulation of L-alanine by the mucosa from a patient with glucose-galactose malabsorption indicate that the energy dependent sodium pump is undamaged. The accumulation of L-amino acids is considered to be dependent on the sodium pump in the same way as the glucose and galactose accumulation (32).

In Csáky's model of intestinal glucose absorption there is also a nonenergy dependent entry step (8). Next comes a sodium-dependent active

transport step which has a higher affinity to glucose and galactose (and some other sugars of similar structure) than to other sugars, e.g. mannose or xylose (8). When the concentration of glucose at the mucosal side is high, the active transport seems to be of little significance. At glucose concentrations of 110 mM at the mucosal side of the rat gut the rate of glucose absorption is unaffected when all sodium ions in the gut content are replaced by potassium ions (9).

Accepting Csáky's concept of glucose absorption it would at first seem reasonable to assume that the active transport step is defective in the patients with glucose-galactose malabsorption. As has been said, the both sugars known to have a high affinity to the active transport system are badly absorbed. Our patients demonstrate a very slow absorption when the sugar concentration at the mucosal side of the intestinal cells is low i.e. when the active transport normally is of significance. In previous observations (21), however sugar absorption was found to be very small also when the glucose concentration of the intestinal content was relatively high—about 60 mM In a routine oral G T T performed with 10 per cent glucose solutions the glucose concentration in the upper intestine is still higher and well above 100 mM Patients with glucose galactose malabsorption have flat G T T curves. This can- be compatible with a defect only at the level active transport according to Csáky's model unless there is a large species difference Hence also with Csáky's concept of glucose absorption the patients with glucose-galactose malabsorption seem to have a disturbance at the level of the carrier-mediated entrance of glucose and galactose into the intestinal cells.

The sodium activation of certain disaccharidases, such as invertase has led Semenza *et al* (34) to suggest that invertase and the glucose-galactose carrier may have a common sodium-binding site. If this theory is correct, and if we assume the carrier transport of glucose not to be functioning in our patients, the finding of normal sodium activation of the invertase suggests that the defect is not at the sodium-binding site of the carrier Also the inability of phloridzin to inhibit further the slow glucose uptake by the mucosa from a patient with glucose-galactose malabsorption makes a defect at the hexose-binding site of the carrier more probable

In the perfusion studies on the adult patient with glucose-galactose malabsorption a secretion of glucose into the lumen of the intestine was found when a glucose-free solution was used. This was apparently caused by a leakage of glucose from the blood. The blood glucose concentration was about 5 mM When a 12 mM glucose solution was perfused, the mean lumen concentration was about 10 mM i.e. an about equally large concentration gradient existed in the opposite direction. This caused an absorption of glucose from the intestine which was equally large as the secretion had been in the other experiment. This may fit with the assumption that in patients with glucose-galactose malabsorption there is no specific mechanism which influences the direction of glucose transport through the intestinal wall, but it has to be remembered that part of the glucose may also originate from more proximal parts of the gastro-intestinal tract.

The relatively high absorption rate of mannitol in the patient is difficult to explain. Since there was essentially no mannitol in the blood the concentration gradient for mannitol across the intestinal wall was about twice the gradient for glucose when glucose was present in the perfusate, but mannitol was absorbed about four times as fast as glucose. In the control subject the mannitol absorption rate was considerably smaller than in the patient and so was the ^{22}Na-absorption rate. These differences probably have a common explanation, e.g. a livelier segmental peristalsis in the patient. The perfusion study gives no evidence for an impaired absorption of sodium in the patient. The sodium absorption is of the same magnitude as found in normal controls by other investigators (16 38)

SUMMARY

Intestinal biopsies from two patients with glucose-galactose malabsorption have been subjected to biochemical and histological studies and the absorption of glucose mannitol and sodium has been studied with a perfusion technique in one of the patients. No accumulation of glucose against a concentration gradient was found during *in vitro* incubation of mucosa from the patients. Phloridzin did not further decrease the slow glucose uptake by the mucosa. L Alanine uptake was normal. Intestinal mucosa from both parents of

a patient with glucose-galactose malabsorption accumulated glucose. In mucosa from patients with glucose-galactose malabsorption disaccharidase activities were normal, sodium activation of invertase could be demonstrated and ouabain-inhibited (pump-) ATP-ase activity was present. The histology was essentially normal both by light microscopy and electron microscopy. The perfusion study *in vivo* demonstrated not only a very slow absorption of glucose but also leakage of glucose into the lumen. Sodium absorption was not impaired. From the data obtained it is suggested that this inborn error of metabolism is localized at the level of the glucose-galactose membrane carrier of the small intestinal mucosa.

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GLYCOSIDASES IN SKIN AND PLASMA IN HUNTER'S SYNDROME

Abnormality of a β-galactosidase in Skin

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INTRODUCTION

Inborn primary disorders of the metabolism of glycosaminoglycans (earlier called acid mucopolysaccharides) can be separated into several different entities (2, 4) usually called the mucopolysaccharidoses (MPS). Among these, type I (Hurler syndrome) and type II (Hunter's syndrome) are best known. The clinical symptoms and signs are quite similar in MPS type I and II. Certain differences do, however exist, but these differences do not always manifest themselves clearly in each single case of MPS. It is, consequently often very difficult, or impossible, to state, whether type I or type II should be diagnosed. Genetically the two entities are distinctly different, type I being inherited in an autosomal recessive mode and type II in a sex-linked recessive mode

A chemical separation of MPS type I and II has been claimed to be possible by some authors (3 8) but others have been unable to demonstrate such a difference (1).

Recently it was suggested that at least some cases of MPS may have low activity of a β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) in liver (5 9) and skin (6) Several other hydrolytic enzymes with pH-optima on the acid side were found to be more active than normal in the tissues (5 6, 9). In neither of the communications were described cases that could definitely be classified as MPS type II For that reason, the results of enzyme assays in two patients with MPS type II will be presented in this paper

Abbreviations: MPS = mucopolysaccharidosis or mucopolysaccharidoses.

MATERIAL

Controls

These will be described in separate publication (6).

Patients

Case 1

Male, 14. Very slowly progressive symptoms including mental retardation, large head with thick skull, coarse facial features with flat nose and large tongue, noisy mouth breathing, hepato-splenomegaly probably cardiac abnormality gibbus, stiff joints in the elbows, hands and fingers, clumsy hands and deformed metacarpals and vertebrae. No definite clouding of the cornea. The patient is still in rather good condition, although easily fatigued. The excretion of glycosaminoglycans in the urine, assayed according to Teller *et al.* (7), was found to be greatly increased.

Case

Male 9 Half-brother of case 1 with the same mother but with different father not related to that of case 1 Similar symptoms and signs as case 1 although slightly less advanced, including also inguinal hernia.

METHODS

Biopsy procedure The skin of the thigh was anaesthetized by nerveblock anaesthesia taking care not to infiltrate the area, from which the biopsy specimen was to be taken. A small piece of skin was cut out and immediately frozen by solid CO

Plasma sampling Blood samples were taken in heparinized tubes in the morning, the patients having fasted overnight. Plasma was rapidly separated at +4 and frozen by solid CO_2.

Enzyme assay These were performed after storage of the samples for 24–48 hours in solid CO_2, using methods described in separate communication (6). All substrates were synthetic. For the assay of β-galactosidase, 4-methylumbelliferyl-β-D-galactopyranoside was used

Table 1 *Enzyme activities in skin*

All alues in µmoles of substrate split/g protein i the homogenate/min

	β-galactosidase	β-glucuronidase	β-acetyl-glucosaminidase	β-glucuronidase / β-galactosidase	β-acetyl-glucosaminidase / β-galactosidase
Hunter s syndrome					
Case 1	0.119	0.9_3 **	14.1	7 76	118.5***
Case 2	0.133	0.233	5.23	1 75	39.3
Mean value	0.1 6(*)	0.578	9 66	4 76**	78.9***
Controls[a] (= 11)					
Mean, ± s.D.	0.390 ± 0.196	0.178 ± 0.140	2.65 ± 1.31	0.475 ± 0.322	7.58 ± 4.46
(Range)	(0.178 − 0.717)	(0 079 − 0 489)	(0.88 − 4.76)	(0.098 − 0.956)	(3.15 − 17 4)

From Öckerman (6).
( )– Values differing significantly from the control values ((*) $p < 0.1$ $p < 0.05$ ** $p < 0.01$ $p < 0.001$).
Not statistically significantly different from the control alues.

RESULTS

Skin

Table 1 demonstrates the activities of three acid hydrolases in the skin. The activity of β-galactosidase in both patients with MPS type II was about one third of the mean value in the controls and lower than the lowest value in any of the controls. In contrast to this, the activities of β glucuronidase (β-D-glucuronide-glucuronohydrolase, EC 3.2.1 31) and β-acetylglucosaminidase (β 2-acetamido- -deoxy-D-glucoside acetamidodeoxyglucohydrolase EC 3 2.1.30) were more than five times higher in case 1 and 30% to 100% higher in case _ than the mean values in the controls.

Thus, a separate enzyme pattern was found in both cases of MPS type II as compared to that in the controls. To illustrate this difference more clearly the quotient of the activity of β-glucuronidase to that of β-galactosidase as well as that of β-acetylglucosaminidase to β-galactosidase was calculated (Table 1) These quotients were about 15 to 16 times higher in case 1 and about four to five times higher in case 2 than the mean values in the controls. In both patients they were higher than the highest corresponding values in any of the controls.

Plasma

In plasma (Table _) significantly higher activities than in the controls were noted for β-glucuronidase and β-acetylglucosaminidase in both patients with MPS type II Significantly increased values were also found for α-fucosidase in case 1 and for β-galactosidase and α-mannosidase (α-D-mannoside mannohydrolase EC 3.2.1.24) in case 2.

DISCUSSION

The results presented in this communication are very similar to those found in skin and plasma in

Table 2. *Enzyme activities in blood plasma*

All alues in µmoles of substrate split/l plasma/min. Statistical significances as in Table 1

	β-galactosidase	β-glucuronidase	β-acetyl glucosaminidase	-mannosidase	α-fucosidase
Hunter syndrome					
Case 1	0.301	0 240**	9 68	_43	7 77*
Case 2	0.502*	0 232	8 68	4.10*	3 80
Mean value	0.40_	0 236**	9 18	3 7*	5 79
Controls[a] (= 1 16)					
Mean, ± s.D.	0._393 0 0676	0 0619 0 0300	5 06 ± 1 8	2 13 0.58	3 62 ± 1 82
(Range)	(0 140 0 335)	(0 0 47 0 1269)	(3 52 7 73)	(1 33 3 0)	(1 34 7 00)

From Öckerman (6)

Table 3

	β-galactosidase	β-glucuronidase	β-acetyl-glucosaminidase	α-mannosidase	α-fucosidase
Skin	0.057	0.407	7.43**	—	—
Mean value case 1–3	0.103	0.521	3.92***	—	—
Plasma	0.322	0.525 **	8.55	3.32	5.04

cases of MPS, definitely of type I, and also in cases classified as either type I or II (5, 6). A similar reasoning on the significance of the findings as the one performed in ref. 6 may be applied here and the conclusion be drawn that the low activity of β-galactosidase is not due to maltreatment or storage of the tissue specimens. The findings would, consequently, support the following conclusion. There exists in skin in MPS type II and abnormality of the pattern of the three glycosidases measured. Possibly a decrease of the activity measured as β-galactosidase forms part of this pathological pattern. More work would, however, be needed to prove the existence of such a decrease. The present results do not allow any statements on, whether there exists any difference in this possible defect of the β-galactosidase activity between MPS type I and MPS type II. Nor is it possible to say if an abnormality of β-galactosidase activity in skin in MPS type II would be caused by a primary defect of some specific β-galactosidase enzyme.

SUMMARY

Two patients with Hunter's syndrome (mucopolysaccharidosis type II) were studied. The exact diagnosis could be settled by the finding of clinical symptoms and signs, typical for this form of mucopolysaccharidosis, and of greatly increased excretion of glycosaminoglycans (acid mucopolysaccharides) in the urine as well as by the fact that the patients were half-brothers with unrelated fathers.

Analyses of glycosidases in skin demonstrated the existence of low activity of β-galactosidase in both patients. β-acetylglucosaminidase was more active in both patients and β-glucuronidase in one of them than in the controls.

In plasma increased activities were noted for β-glucuronidase and β-acetylglucosaminidase in both patients and of β-galactosidase and α-mannosidase activities in one of them and of α-fucosidase activity in the other.

The results are very similar to those described in patients with Hurler's syndrome (mucopolysaccharidosis type I).

ADDENDUM

After the manuscript was accepted for publication skin and plasma, kindly supplied by Dr S. L. Bjorklund, could be studied from one further patient with Hunter's syndrome.

Case 3: male, 19. Very slowly progressing symptoms, including shortness of stature, large head, coarse facial features with large tongue, uneven teeth, short neck, stiff joints, deformities of the thoracal skeleton, inguinal hernia and impaired hearing. No definite clouding of the cornea, no obvious mental retardation, no hepatosplenomegaly. Four sisters, all healthy. Markedly increased excretion of glycosaminoglycans in the urine. The results noted in this patient are given in Table 3.

All findings in case 3 give further support to the conclusions drawn in this paper. By adding the value in skin in case 3 the significance of the decrease of the β-galactosidase activity was increased ($p < 0.05$).

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A COMPARISON OF THE EFFECTS AND SIDE EFFECTS OF PENICILLIN V AND AMPICILLIN IN THE TREATMENT OF SCARLET FEVER

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Penicillin V is extremely efficacious against infections caused by haemolytic streptococci. A comparison with the broad-spectrum penicillin, ampicillin, is justified since this drug, too, has a highly effective bactericidal action on pyogenic streptococci. On the basis of a small series of cases in France ampicillin has even been considered "l'antibiotique de choix for the treatment of scarlet fever (1). On the other hand a fairly high incidence of side-effects is reported from the use of ampicillin, especially in the form of exanthems (3 4 5 6) which may be a restraining factor An aetiologically and clinically uniform disease such as scarlet fever should be a suitable object for the study of the antibacterial and clinical efficacy of ampicillin as well as of its side-effects in comparison with penicillin V our hitherto best per oral penicillin for scarlet fever therapy

MATERIAL AND METHODS

The study covers the period 196[illegible]–64 The patients were treated in the same department. As from the morning of the second day in hospital alternate cases of scarlet fever [illegible] ere put on ampicillin (Doctacillin)—dose 125 mg 3 for ages 1–4 years and 250 mg 3 for ages 5–13 years—and penicillin V (Åkrepenam)—dose 100,000 IU (60 mg) 3 and 200,000 IU (120 mg) 3 for the two age groups The treatment lasted for 10 days. Daily tests for haemolytic streptococci were made during the 10 days in hospital and thereafter at follow-ups one and three week after discharge. The antistreptolysin titr of children above 4 years of age was determined on admission, after one [illegible]eek and at the second follow-up [illegible] about [illegible] eeks after discharge. Each series consisted of 110 cases

RESULTS

Clinical effect The results were satisfactory with both drugs No complications occurred, either purulent or of toxic-allergic origin in the form of myocarditis or nephritis.

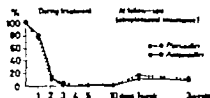
The *duration of the fever* as from the onset of the disease was for the penicillin V series 4.2 ± 0 16 days and for the ampicillin series 4 6 ± 0.[illegible]3 days, and during the period in hospital 1 9 ± 0 10 and 2.3 ± 0 19 days respectively—thus a rather shorter duration for the penicillin V patients, but not statistically significant.

The *sedimentation rate* on admission to hospital and one week later was for the penicillin V series 28.9 ± 1 4 and 11 6 ± 0 7 mm/hr respectively and for the ampicillin series 28.3 ± 1.3 and 11 4 ± 0 7 mm/hr thus practically no difference

Bacteriological [illegible]ffect This is illustrated in Fig. 1 which includes only those patients who had a positive test on the first day (98 in the ampicillin, 93 in the penicillin V series) All of the penicillin V series were negative on the third day In the ampicillin series two patients did not become free from bacteria until the fourth day and one not until the fifth. Streptococcal recurrence occurred rather more often in the ampicillin patients at the check-up after one week (15 4% against 9 1%). The difference is not significant. During the follow-up period pharyngeal symptoms accompanied by fever were noted in 1[illegible] ampicillin cases and in 3 penicillin V cases, of whom 7 and 3 respectively with streptococci in the pharynx.

Serological reaction The antistreptolysin titre was determined in 63 ampicillin cases and 62 penicillin V Twelve and eleven cases, respectively had no rise of titre. The mean titres for the two series were almost identical (161– 65–338 and 160–[illegible]60–334)

Recurrence of scarlet fever During the observation period, lasting 3 to 4 [illegible] years, 3 in each series has had a [illegible]ecurrence of scarlet fever one


Fig 1 Streptococci in pharynx.

patient within only one month, the remainder 10 months – 2 years 10 months after the first attack.

Side-effects. Reactions were noted in a considerable number of the ampicillin cases. No less than 10 patients (9%) developed *exanthema* on the eighth to tenth day In two cases of fine papulous type, in the remainder in the form of small spots, in three of the latter the extremities were affected, in the other cases both the trunk and extremities, usually in a symmetrical pattern. In three patients the exanthema ultimately covered the whole body being confluent and of a bright reddish cyanotic colour The *conjunctiva* were in three cases moderately swollen and reddened, in one case being heavily inflamed with at the same time a spotty *enanthema* on the palate and buccal mucosa and sores on the lower lip Six cases had a moderate fever 37 .5–38 3C The symtoms disappeared within a day or two after discontinuation of the drug after the tenth day

Another observation was also made In no less than 11 cases (10%) the fever returned after the patient had become afebrile without any clinical explanation. The rise of temperature occurred in one case on the third day in two on the fourth, in two on the fifth, in one on the sixth, in four on the seventh, in one on the eighth, and reached a peak of 37 .8C in one patient, 38 –38 9C in six, 39 –39 6C in four Remarkable, too, was that in most cases the temperature showed a spontaneous tendency to return to normal, and the fever disappeared as soon as administration of the drug was stopped after the tenth day The sedimentation rate showed no rise in these cases in comparison with the remainder of the series. Two patients had diarrhoea for a few days, one accompanied by *vomiting*, *which also affected* another patient.

In the penicillin V series there was no case of exanthema. Four patients had a rise of temperature to 38 –38 4 one on the fourth, two on the sixth, and one on the ninth day without any clinical explanation

DISCUSSION

Since many years we have with very good results treated scarlet fever with penicillin V in the dosage used in this series. For ampicillin we prescribed a dose in accordance in the main with the recommendation suggested for treatment of infections due to Gram-positive bacteria. In that way ampicillin was given in twice the quantity of penicillin V

The results of these treatments show that both types of penicillin were very effective. Clinically they were equally efficacious during the primary phase and no complications occurred The fever lasted only slightly longer in the patients treated with ampicillin. Bacteriologically too the drugs were similar in their effects in some of the ampicillin cases the haemolytic streptococci disappeared rather later from the pharynx The similar result of treatment is seen also in the serological response with an identical course of the anti-streptolysin curve. In the after-course streptococcal recurrence occurred to a rather greater extent in the ampicillin cases, and tonsillitis due to streptococcal recurrence was more frequent than in the penicillin V series. Besides, when taking into consideration that the dose of penicillin V was half that of ampicillin it can be asserted that penicillin V was superior in respect to bactericidal effect.

Immunity to scarlet fever is weakened by penicillin therapy as I reported in 1954 (9). On the bases of the present material the recurrence within the period that has elapsed is equal for the two series (about 3)

As regards side-effects the present study showed a very great difference between the two types of penicillin No immediate or severe reactions were likely with the per oral medication, though late

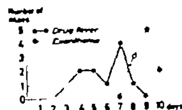


Fig Reactions in patients on ampicillin Type and time of onset.

r‍· ions of serum disease type were more to be ‍‍... ‍‍‍‍‍‍...d With penicillin G and V however these ‍‍‍...actions are uncommon. In combination with probenecid, on the other hand fever and exanthema occur and mucocutaneous symptoms are found in a high percentage of cases as I have ...own previously (8) In that context, too a mucocutaneous syndrome was brought on in a ld by means of a provocation test with penicillin V (10).

In the present material, however penicillin V caused no case of exanthema. No less than 10 cases, on the other hand occurred in the ampicillin series, in six accompanied by a moderate fever 37 .5–37 .8 one case 38 .2. Mucous processes were also provoked, in three cases conjunctivitis and in one a general, intense reddish cyanotic ...anthema over the entire body combined with conjunctivitis and stomatitis and moderate fever ...sting 3 days, i.e. a relatively mild but distinct febrile mucocutaneous syndrome of the type usually denoted Stevens-Johnson's syndrome in the Anglo-Saxon literature

A very remarkable observation is the febrile condition which occurred in no less than 11 cases (10%) of the patients on ampicillin after the disappearance of the initial fever There was no clinical explanation nor rise of sedimentation rate in these cases. There were, however four similar though less pronounced cases of a rise in temperature in the penicillin V series.

The first question is whether the two forms of reaction are of the same or of different types. One may assume that cases of fever alone re present preliminary stage and the combination with exanthema a more advanced stage But Fig. 2, in which the times of onset are plotted, shows a manifest dissimilarity In cases of fever alone the reaction comes at an earlier stage of the treat ment, on the third to eighth day (only one case on the 8th), compared with the eighth to tenth day in the exanthema cases In four of these cases, moreover fever was lacking, and n the remainder was low very much lower than in the febrile cases. If the same mechanism wer involved, cases with high fever should be especiall often combined with a rash.

The exanthema cases should be comparabl to serum disease, i.e. of immunological or gin and eferable to allergic reactions. There is no doubt that in that respect ampicillin is allergenicall very much more potent drug than penicillin V On the other hand one has to take into consideration that the high percentage of allergic reactions in the ampicillin cases may depend upon the combination with streptococcal disease The underlying disease is of importance (8 10)

The purely febrile cases must have a different genesis. They must represent some non-allergic type of drug fever ( ). Some drugs may cause fever probably by direct action via the central nervous system As regards the febrile cases a common factor would seem to exist in the tw penicillins even if ampicillin produced the stronger reaction (higher dosage!) I seems hardly likely that there is any factor in the penicillins which is directly provocative of fever An effect m ght conceivably be produced by a change in the intestinal flora, resulting in breakdown of Gram-negative bacteria and giving rise to a pyrogenic substance (6, 7). Further studies may perhaps cast light on this problem. From the practical point of view however it may be of alue to know that ampicillin in ordinary dosage may give rise to fairly pronounced fever which admittedly does not seem to be of a serious nature for the patient but may lead to a wrong diagnoses or to a wrong assessment of the results of treatment

SUMMARY

A comparison of the effect of penicillin V and ampicillin in the treatment of scarlet fever has been made in two series of 110 patients each. Clinically and bacteriologically both drugs showed very satisfactory effect, penicillin V being superior—half the dose of this drug bringing about rather shorter period of fever quicker elimination of bacteria, and less streptococcal re currence. The serological reaction, judged from the antistreptolysin titre was the same in the two series, and likewise the incidence of recurrences of scarlet fever

As egards si‍‍‍ -effects there was a considerable difference Exanthema, somet mes accompanied by fever occurred on the eighth to tenth day of 10 ampicillin cases (9%) but in no penicillin V case One patient on ampicillin had a mild mucocutaneous yndrome In 11 (10) of the ampi‍‍‍...illin cases and 4 of the penicillin V cases ther w s a clinically inexplicable febrile condition of fairly pronounced nature beginning on the third

to eighth day The author interpret the exanthema cases as an allergic reaction of the nature of serum disease the febrile cases on the other hand as a non-allergic type of drug fever perhaps being brought about by a change in the intestinal flora. A knowledge concerning the latter type of reaction is of practical significance.

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HISTAMINURIA IN HARTNUP DISEASE

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The basic abnormality in Hartnup disease is supposed to be a defect in the transport of monoamino-monocarboxylic amino acids both in the renal tubules and in the small intestine (6). This hypothesis is based mainly on studies of tryptophan metabolism, since other amino acids have been studied only sporadically. Malabsorption of tryptophan with ensuing increased bacterial, intraluminal degradation of tryptophan to indoles has been considered to explain the abnormal indole excretion in this disease (11).

Recently one of us (O. S.) has studied some aspects of histidine-histamine metabolism in healthy individuals and in patients with dystrophia myotonica (15–19). These studies demonstrate that in patients with dystrophia myotonica increased excretion of conjugated histamine in the urine coexists with increased levels of histamine like activity in the faeces. The basis for the increased urinary output of conjugated histamine seems to be an increased intraluminal production of histamine-like activity in the large bowel (18). A plausible explanation of this finding would be that these patients had malabsorption of L-histidine. However, it was demonstrated that there was no appreciable malabsorption of this amino acid when administered alone (5).

These studies thus questioned the view that increased urinary excretion of metabolites formed by bacterial degradation of amino acids in the gut, is caused by malabsorption of the respective amino acid. It was, therefore, deemed of great interest to study the same aspects of histidine histamine metabolism in Hartnup disease, a disease in which malabsorption of histidine according to present theories should form an integral part.

MATERIAL

Two siblings with Hartnup disease were studied. Both, N. A., a girl aged 18, and E. A., a boy aged 14, have been described in detail elsewhere (4).

Both receive nicotinamide, 40 mg daily, also at the time of study, and clinically their condition has improved over the last years, N. A. maintaining a full time job as hairdresser, and E. A. finishing his 7th year at school.

The tests were carried out in the course of approximately 1 year.

METHODS

Free and conjugated histamine in urine. Urine was collected as 24 hour samples, and histamine was estimated by Duner & Pernow's method (2, 16), which involves ion-exchange and bioassay on the isolated guinea-pig ileum. No cultures of urine were carried out in our patients during these experiments.

Free histamine in the faeces was estimated as described by Anrep et al. (1, 7). Three samples were also examined with a more specific method (17). Except where specifically stated otherwise, *conjugated histamine in the faeces* was determined as previously described (method ... (19)).

Faecal incubations with L-histidine were carried out as described in detail elsewhere (18). The specimen was mixed thoroughly in a mortar and portions of 5 g were mixed with 15 ml distilled water and 300 mg L-histidine monohydrochloride monohydrate (Sigma). The sample was incubated aerobically at 37°C for 18–20 hours.

An antihistaminic agent (Allergan®) was occasionally applied to the bath of ileum and antagonized the biological activity of the extract and of equiactive doses of authentic histamine diphosphate (Nutritional Biochemicals Corporation) to the same degree (13). All values for histamine are recorded in terms of the base, and no corrections have been made for losses during the procedure.

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Table 1 *Urinary and faecal excretion of free and conjugated histamine in 2 patients with Hartnup disease*

Urinary excretion is given as μg base/24 hours; faecal excretion as μg base/g wet weight

	Urine		Faeces	
Initials	Free	Conj.	Free	Conj.
N A	28	170	0.2	—
N A	49	130	0.2	—
N A.	19	63	0.9	4.6
N A.	12	78	0.2[a] 0.2	3 4
N. A	—	—	71	—
E. A.	10	1 300	—	—
E A	15	930	—	—
E A	9	1 000	1 9[a] 2.5[b]	0.5
E A		400	0.2	—
Control series[c]	12.6 ± 6.3	30.0 ± 23 6		

[a] Two defaecations on same day
[b] Total (free + conjugated) histamine in μg/g wet weight estimated as described elsewhere (17).
[c] Mean excretion ± S D.

pH in the faeces and in the incubation mixtures was measured potentiometrically (19)

Indican excretion in urine was measured as described by Sharlit (14) and by Meiklejohn & Cohen (9), except that 100 ml 1.2 *N* hydrochloric acid were used as preservative instead of toluene.

Loading experiments with L-histidine were carried out in the following way The patients were kept on a standard diet, each meal consisting of 2 slices of whole meal bread with cheese and 400 ml of milk. This diet was given days prior to loading, on the day of loading, and on the two following days, and the meals were given at 8 15 a.m., 1 15 p.m. and 5 30 p.m. Urine collections started at 8 a.m. The oral dose of L-histidine monohydrochloride monohydrate (10 g per 70 kg body weight) was given with approximately 100 ml tap water just before the first meal

RESULTS

Excretion of histamine in urine and faeces

The mean excretion and range of urinary free and conjugated histamine in a control series (16) were 1_6 (2 31) and 30.0 (1–99) μg base/ 4 hours, respectively (unweighted values).

The results in our patients are summarized in Table 1 The mean excretion of conjugated histamine in both patients was above the upper normal limit, whereas the mean excretion of free histamine was within the normal range. A single value in N A. was above the normal range.

The histamine-like activity in the faeces seemed to be moderately elevated in both patients (Table 1) (according to Jiménez Díaz *et al* (7) the mean value for males is 0 018 (0 03 histamine hydrochloride) and for females 0.27 μg base/g). From the experience with patients suffering from dystrophia myotonica, 1000 μg conjugated histamine in the urine would usually correspond to 20–30 μg/g wet weight in the faeces. The excretion in faeces in E. A. was thus less than would be expected from the experience with dystrophia myotonica. The value of 71 μg/g wet weight in N A indicates that a considerable abnormality may exist also as for faecal histamine-like activity in patients with Hartnup disease.

Two samples from N A. were tested for stability of the faecal histamine-like activity at room temperature (24 hours) In one sample the content increased from 0.2 to 0.4 in the other it decreased from 0._2 to 0.12 μg/g wet weight.

The water contents in three samples from each patient averaged 69%, (range 59–74%). Faecal pH in one sample from E. A. was 6 7 in two samples from N A. the values were 7._5 and 7.5 (normal range 6.5–7.8 (19)).

The 4 hour urinary excretion of indican (corresponding to the first histamine values for each patient, Table 1 was 94 mg (N A.) and 11 mg (E. A) In a control series, the mean and range of indican excretion were 62.9 (41 113) mg/ 4 hours (19).

Loading experiments

The increase in urinary free histamine during the first 24 hours after histidine loading, regularly encountered in healthy individuals (8, 1_ 15) seemed to take place in these patients as well (Table _). However the spontaneous variation (Table 1) in urinary excretion of free histamine impedes the interpretation of this finding.

There also seemed to be an increment in the urinary excretion of conjugated histamine on the day of loading When considered alone, the increment in urinary conjugated histamine in E. A. may seem doubtful. When considered together with the increment in the free faecal fraction it seems plausible that the increment in urine is real.

Formation of histamine in the faeces

It may be seen from Table 3 that histamine is formed in samples from both patients, the forma

Table 2. *Urinary and faecal excretion of free and conjugated histamine after an oral L-histidine load*

		Control		Loading		
Initials	Day No. ...	1	2	3	4	5
N. A.	Urine Free	19	12	36	22	48
	Conj.	63	78	240	82	67
	Faeces Free	0.9	0.2[a] 0..	0.7	1.4	0.2
	Conj.	2.6	3.4	3.4	0.8	—
E. A.	Urine Free	9	2	18	6	8
	Conj.	1000	400	590	110	93
	Faeces Free	1.9[a] 2.5	0..	—	70[c]	..1
	Conj.	0.5	8.8	—	—	

[a] Two defaecations on same day.
[b] Total (free + conjugated) histamine, estimated as described elsewhere (17).
[c] A total of 3.3 mg histamine base in the faecal sample.

tion being most pronounced in an acid environment (3). Whereas the formation in faeces from E. A. was pronounced—at pH 4 there seemed to be almost complete transformation, provided the biologically active substance is identical with histamine—the formation in faeces from N. A. was moderate, although definitely larger than that usually found in faeces from healthy individuals, especially at pH 4 (18).

The stability of the *formed* histamine was not tested.

DISCUSSION

In Hartnup disease, increased levels of conjugated histamine in urine seem to coexist with moderately increased levels of histamine-like activity in faeces.

On incubation, especially in an acid environment, histamine was formed from L-histidine in faecal specimens from patients. It therefore seemed likely that, after oral administration of L-histidine, the unabsorbed fraction would partially be converted to histamine in the lumen of the large bowel. Nevertheless, only moderate intraluminal histamine formation seemed to follow the ingestion of L-histidine in our patient. In at least one patient with dystrophia myotonica (15) the increment in faecal and urinary histamine after oral L-histidine loading was larger.

There may be several causes for the observed

Table 3. *Histamine formation in 5 g faecal samples after the addition of 300 mg L-histidine monohydrochloride monohydrate (222 mg of the free amino acid)*

Experiments between solid horizontal lines were carried out with the same faecal specimen.

		pH of incubation mixture		
Initials	Inherent faecal histamine µg/g	Start	End	Additional histamine after incubation (µg/sample)
N. A.	0	7..	7.8	130[a]
N. A.	0	6.2	6.6	180[a]
N. A.	0.22	7.0	—	67
N. A.	0.22	6.0	—	96
N. A.	0.22	4.0	—	3 400
E. A.	0.2	7.0	—	8,800[b]
E. A.	0	5.7	—	56,000[c]
E. A.	0.2	4.8	—	10 000[c]

[a] The contractions caused by these extracts and equiactive doses of authentic histamine were counteracted to the same extent by an antihistaminic agent (Allergan®).
[b] Conjugated histamine 140 µg base.
[c] Conjugated histamine was not definitely present.

lack of intraluminal histamine formation in Hartnup disease. The intraluminal formation of histamine may be less than the *in vitro* formation in the faeces. Formed—especially intraluminally formed—histamine may be less stable than the spontaneously occurring histamine in faeces. Only a small fraction of the ingested L-histidine remains unabsorbed. The most probable explanation seems to be the last-mentioned one.

Milne (*10*) has set forth five criteria to assess an intestinal absorption defect of amino-acids in a given disorder After ingestion of the amino-acid to be tested, there should be a lesser increment in plasma levels than occurs in control individuals (*a*), and more of the amino-acid should appear unchanged in the faeces (*b*) Likewise, after ingestion of the amino-acid, larger quantities of products of intraluminal bacterial degradation should appear in the faeces (*c*) as well as in the urine (*d*), as compared with control individuals. Finally unusual bacterial strains, stable on subculture, capable of degrading the amino-acid may be found in the faeces (*e*).

The present study shows that three of these criteria, viz. (*c*), (*d*) and (*e*), seemed to be present as for orally administered L-histidine in Hartnup disease. It should be noted, however that the subculture qualities of faecal samples have not been tested. This seemingly substantiates the view that Hartnup disease there is malabsorption of L-histidine (6) in addition to that of tryptophan (11 21).

Nevertheless, the very same three criteria are present as far as L-histidine is concerned in patients with dystrophia myotonica (18, 19), but in the latter disease there is no appreciable malabsorption of L-histidine when administered alone (5). By analogy the presence of these three criteria probably does not suffice to ascertain that malabsorption of L-histidine is present in Hartnup disease. Accordingly it seems indicated to test whether malabsorption of L-histidine really is present in Hartnup disease. Such an investigation has been carried out (Halvorsen, Hygstedt, Jagenburg & Sjaastad, to be published).

SUMMARY

Two patients with Hartnup disease showed increased urinary excretion of conjugated histamine and increased faecal excretion of histamine-like activity After addition of L-histidine, histamine was formed in faecal specimens *in vitro* After ingestion of L-histidine there were signs of no more than a moderate intraluminal formation of histamine in the large bowel. These findings indicate that in Hartnup disease there is no marked malabsorption of L-histidine, a thesis that will be tested further

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CLINOCEPHALY

Considerations on the pathophysiology of Craniosynostosis

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Craniosynostosis is not a constant condition but "primarily an endless, varying disease of infancy" (9) However it is for practical purposes necessary to group the cases and currently the most used classification is the following: brachycephaly (tower head) scaphocephaly (boat-shaped head), plagiocephaly (oblique head) and trigonocephaly (triangular head). To these true forms of craniosynostosis comes three symptom complexes. Crouzon's disease (6) Apert's disease (5) and Carpenter's syndrome (1) where the head deformity is of the brachycephalic type. We certainly agree with Laitinen (9) that this classification is enough, but still we would like to discuss one of the older sub-groups, namely clinocephaly because this type of deformity might have an implication upon the pathofysiology of craniosynostosis. A case with a saddle-like depression transversely of the skull in the region of or just behind the coronal suture, was described by Sömmering (11) and he believed that it was the result of partial closure of the sagittal suture Virchow (13) who coined the term clinocephaly defined it as synostosis of the sfenoparietal suture Backman (4) in his extensive paper on cranial deformities also discussed the saddle-head" and he postulated that synostosis of the sagittal suture as a rule starts at obelion which is the midpoint of the suture. Of his cases were 7% only synostotic in the middle part of the suture, 74% had total obliteration of the sagittal suture and the remaining 19% had varying degrees of premature closure. Almeida & Barros (1) did also find some patients with a transverse bone depression at the middle of the sagittal suture corresponding to the place, where the suture had started to close. McLauren & Matson (10) describe a case in which premature closure of the sagittal and the right coronal suture gave the same skull deformity in the region of the closed coronal suture.

We would like to report and discuss two unusual cases, obviously resulting from partial closure of the sagittal suture.

CASE REPORTS

Case 1

Male. Only child. Mother ged 32. Pregnancy and delivery uneventful. Signs of scaphocephaly among the relatives. At 3 months of age admitted to hospital for investigation of abnormal shape of the head. Cranial X-ray showed no definite proof of premature closure of the sutures (Fig. 1). New investigation at 6 months of age showed a child with normal development and neurological examination was normal. His head was scaphocephalic with a peculiar saddle-like depression in the middle of the head, transverse the sagittal suture (Fig. 2). A new cranial X-ray showed premature synostosis of the midpart of the sagittal suture, corresponding to the saddle-like depression (Fig 3). The child was operated with linear craniectomy The suture was found to be synostotic from point 2 centimeters posterior to the large fontanelle t   point 4 centimeters cephalad the lambdoidal suture. Linear craniectomy was performed of the synostotic part and the shape of the head was restored ith help of small transverse linear craniectomy in the midpart of the synostotic suture Teflon foil as wrapped over the bone edges.

Three months after operation the saddle-like depression had almost vanished and the configuration of the head was nearly normal (F g. 4). One year late the child as normally developed and had normal head (Fig. 5).

Case

Second of two children. Pregnancy and delivery uneventful. Admitted for investigation of suspect V.D.C. at six months of age. A scaphocephalic head ith a

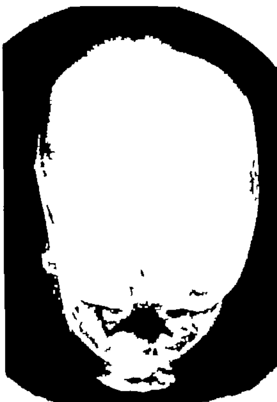


Fig 1



Fig 2



Fig 3

saddle-like depression transverse the sagittal suture was noted. Cranial X-ray showed partial fusion of the sagittal suture. The child as operated upon with linear parasagittal craniectomy parallel to the synostotic part of the sagittal suture, reaching from point just behind the large fontanelle to 3–4 centimeters cephalad to the lambdoidal suture. Teflon foil was interposed.

At the age of one and half year the shape of the head was close to normal and the psycho-motoric development normal.

DISCUSSION

Small bridges of bone across the suture line might possibly disturb the normal development of the



Fig 4



Fig 5

skull (7 8 9). It is also well known that new formed bone bridging over the bone defects in operated cases of premature synostosis impairs the result of the operation. The two reported cases with a partial closure of the sagittal suture are interesting because they show exactly the type of deformity one would expect, if one accepts the theory that bone-growth takes place in the sutures and that there will be a compensatory growth of the rest of the skull if a suture closes prematurely Our cases show underdevelopment of the skull in a direction axial to the closed part of the sagittal suture and a compensatory growth of the rest of the skull One theory regarding the etiology is that there are in the suture lines ossification centers from where the closure starts. If that is true resection of just this part may cure the disease. Furthermore if a part of the suture has closed it is possible that due to apposition of the bone edges in the rest of the suture this closes too as has been suggested in cases of "iatrogenic craniostenosis (2) After resection of the closed part of the suture in our cases the appearance of the skull returned to normal. The facts that only the obliterated part of the suture was resected and the remodeling of the skull still was satisfactory and that the rest of the suture did not close after the operation might be a clue to a solution of the mystery of craniostenosis.

These aspects support the view that all cases of craniosynostosis should be operated and operated upon as early as possible (3) especially as there is a possibility in some cases to perform a smaller operation with resection of just the closed part of the suture

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SERUM INSULIN LEVELS IN CHILDREN DURING GLUCOSE TOLERANCE TESTS

D B Grant

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Low serum levels have been reported in young children after an overnight fast (3 6). In an attempt to investigate the possibility that these low fasting values might reflect low levels of circulating insulin under a variety of physiological conditions in early childhood, immunoreactive insulin was measured during glucose tolerance tests performed on children who had been admitted to hospital. As it was not possible to study healthy children, the investigation was restricted to children who were receiving diagnostic tolerance tests. The results in 18 children who showed no evidence of malabsorption or impaired carbohydrate tolerance are described in this paper

SUBJECTS AND METHODS

10 boys and 8 girls between the ages of 5 months and 13 / years were studied. The weights and heights of the children lay between the 3rd and 97th centiles (7). The subjects' diagnoses are given in Table 1 Although 6 of the children were admitted with history f diarrhoea, the absence of abnormalities either on physical examination or on investigation and the absence of diarrhoea after admission to hospital were *considered to exclude* malabsorption. None of the *subjects appeared ill* at the time of study and none had family history of diabetes mellitus. All the children had normal appetites and had received normal diet, in which at least 40 per cent of the caloric intake was given as carbohydrate, for at least 3 days before the tolerance tests.

The quantity of glucose used in all the tests was calculated from the formula (9)-

$$\text{Glucose (g)} \quad \frac{(\text{body eight (lbs)})^{1}}{(140)} \quad 50$$

This formula gives dose of glucose which is roughly proportional to body surface-area and, as result, the smaller children received larger quantity of glucose per unit body weight.

All the tests were carried out after an overnight fast of 8–14 hours. Following the collection of single fasting capillary blood specimen, glucose as given by mouth as 10% solution. Five further capillary samples were then collected at 30 minute intervals.

Blood sugar was measured by Wilkinson (9) modification of the method of Folin and Wu and serum-insulin was estimated by modification of the radio-immunoassay described by Morgan & Lazarow (5). Details f this assay have been given elsewhere (3). The lowest concentration f insulin which could be detected with any certainty in this assay was estimated to be between 2 3 μU/ml and serum-insulin results lower than this level are expressed as 3 μU/ml.

The significance of differences between mean values was determined by Student *t* test.

This work as supported by grant from the Medical Research Council.

RESULTS

Blood Sugar

The blood sugar results for the 12 children under the age of 3 years are compared with the results for the 6 older children in Fig 1 Although the mean blood sugar levels for the younger subjects were lower than the corresponding values for the older children both before and after the administration of glucose, the difference was significant only 60 minutes after glucose ($p < 0.0?$).

In ‿ f the younger subjects and one of the older subjects, the blood sugar had not returned to the fasting level at the end of the test.

Serum-Insulin

The serum-insulin results for the children under the age of 3 years are compared with the values found in the older children in Fig. 1 The mean insulin levels for the younger children were significantly lower than the corresponding values for the older children both before the administration of glucose ($p < 0.01$) and 30 ($p < 0.005$) and 60 minutes ($p < 0.001$) after the glucose load.

HYPOPITUITARY DWARFISM

The Importance of Early Therapy

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In older children, the diagnosis of hypopituitary dwarfism usually poses few problems with the diagnostic facilities available in most university hospitals today But it has been repeatedly demonstrated that retardation of growth in pituitary dwarfism may be marked in infancy The diagnosis should be made as early as possible because delay in therapy may deprive the child of several cm of height. Growth velocity is great in the first three years of life, and it seems to be difficult to catch up on lost height when therapy is started later in childhood.

Familial occurrence of hypopituitary dwarfism is now generally accepted and heredity as a cause of pituitary dwarfism is well established (7 8 10). Two siblings with hypopituitary dwarfism were admitted to the Children's Hospital University of Bergen. They have been followed from birth up to the age of 6 and 7 1/2 years respectively A preliminary report is published elsewhere. The main purpose of this paper is to demonstrate the effect of long-term treatment when treatment was initiated at the age of 2 and 3 1/2 years respectively

CASE REPORTS

Case 1

E. J is a male child born on June 16th, 1960 His father died from diabetes mellitus in 196–. There is no known case of diabetes, dwarfism or other endocrine disturbances in the father's or the mother family A younger sister of the patient suffers from pituitary dwarfism (Case 2). A three-year-old half-sister is of normal height for her age.

The patient was the product of an uneventful pregnancy and spontaneous delivery at term, weight 4000 g, length 48.5 cm. He was transferred to the Children Hospital when one day old because of shivering. Fasting blood sugar was not determined. No abnormalities were discovered during his stay in hospital. The mother observed marked growth retardation at about six months of age. Psychomotor development was normal.

On his second admission to the Children Hospital, March 21 1961 at the age of 9 months, his height was 59 cm and weight 6560 g. Clinical examination revealed small, well-proportioned male infant. His sex chromatin was negative. Skeletal age averaged 6 months. Some laboratory data are given in Table 1 At this and the following admission at the age of 21 months, no definite diagnosis was established.

In June 1963 at the age of 3 years, he was readmitted for a more complete study He had grown 11 cm in the previous 15 months, and his height was only 69 cm, weight 7000 g. On physical examination he appeared to be a short, well-proportioned male child with small face (Fig. 1). His skeletal age corresponded to 2 years, according to Greulich & Pyle (2). X-ray of the sella turcica was normal. The fasting blood sugar in June and November ranged from 3 to 75 mg per 100 ml. An oral glucose tolerance test was diabetogenic, with a peak of 199 mg per 100 ml but control curve one week later revealed normal rise to maximum of 123 mg per 100 ml. A third glucose tolerance test was performed in 1964 in therapy-free period. This also revealed normal rise in blood sugar concentration, but the patient had hypoglycemic reaction 3 hours after the glucose load. The serum inorganic phosphorus was below normal. There was no increased urinary excretion of 17 KGS following intravenous Metopirone (Ciba). The clinical, radiographic and laboratory (Table 1) findings indicated the diagnosis of hypopituitary dwarfism and the patient was readmitted 4 months later for treatment with human growth hormone (HGH).

Case

M. J the younger sister of Case 1 was born on November 19 1961 Pregnancy was uneventful, delivery uncomplicated, birth weight 3830 g, length 50 cm. Her psychomotor development was normal but growth had been retarded since early infancy

On admission to the Children Hospital, March 29 1962, at the age of 4 months, she measured only 53 cm.

Tale 1 *Laboratory data 1962–63*

Case no.	Year	Hb (g/100 ml)	Fasting blood sugar (mg/100 ml)	Phosphorus (mg/100 ml)	Creatinine (mg/100 ml)	Cholesterol (mg/100 ml)	PBI (μg/100 ml)	Response to intravenous metopyrone
1	1962	9.8		3.2	0.9			
	1963	9.9	3 75	2.8	1.0	130	4.9	Poor
2	1962	10.8		4.2	1 1			
	1963	9 6	43–55	3 3	1.0	146	4 7	Poor

weight 4600 g. The skeletal ge corresponded to her chronological age.

Some laboratory data are given in Table 1 The serum inorganic phosphorus was 4.2 mg per 100 ml. Her growth retardation was initially considered to be constitutional.

On June 10 1963, at the age of 19 months, she was readmitted for more complete studies. She had grown 9.5 cm in the previous 15 months, and height was only 62 5 cm, eight 5300 g. She had similar appearence as her brother (Fig. 1). X-ray of the sella turcica was normal. Here too, the skeletal age corresponded, ac cording to Greulich & Pyle (2), to her chronological age. The fasting blood sugar was constantly low (Table 1). An oral glucose tolerance test revealed delayed fall in

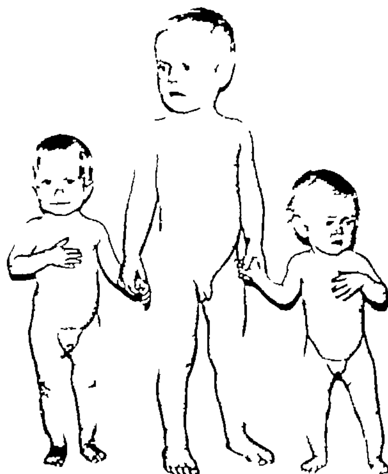


Fig 1 The tw siblings ith pituitary dwarfism at the age of 1 and 3 years, and normal-sized 3-year-old boy

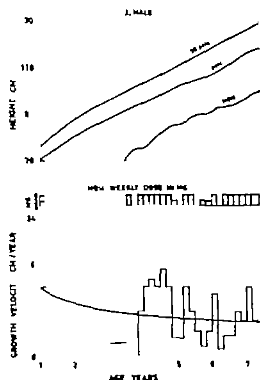


Fig 2 The influence of HGH therapy in Case 1 on growth curve and growth elocity

the blood sugar level, 154 mg per 100 ml after 3 hours. Four other glucose tolerance tests were ithin normal limits. The serum inorganic phosphorus was below normal this time (Table 1). The patient failed to respond t intravenous Metopirone (Ciba) test. The clinical and laboratory data (Table 1) indicated pituitary dwarfism, and she was readmitted four months later for treatment.

Treatment of Cases 1 and 2

Treatment with HGH (Roos *et al* 1963) 2 mg three times weekly and desiccated thyroid (Nyco), 0.3 g daily was started in the siblings on December 3 1963 at the age of 3 1/2 and 2 years respectively (Fig. . and 3). Therapy had to be discontinued . months later owing to febrile reactions 10–1. hours after each injection and ultimately enlarged li er and spleen in both patients. An excellent response to HGH therapy (Figs. . and 3) confirmed the diagnosis of pituitary dwarfism. On March 16th, 1964 the same treatment was re-initiated. The HGH used this time was prepared by Trygstad according to a modification of a method employed by Roos, Fevold & Gemzell, and the ad'pokinetic effect of this preperation was negligible (9). No untoward reactions to this preparation have been observed. Total height increase in the siblings during a 4-year-period was 29.5 and 32.5 cm respectively

A reduction in the dosage of HGH to 1 mg three times weekly to each of the two siblings, for three periods of 2 months resulted in a reduction in growth to a total of 1.5 and 1.8 cm in 6 months. Very little growth was observed in the periods without therapy (Figs. 2 and 3). The same rate of growth was observed in a period without additional thyroid therapy as when desiccated thyroid was given.

Special studies in 1965–67

Insulin tolerance was not investigated initially owing to fasting hypoglycemia. Increased insulin sensitivity was demonstrated in 1966, and the hypoglycemia gave no increase of growth hormone in plasma (Table 2). (The high plasma growth hormone level in the boy in 1965 may have been due to technical error) (Table). All plasma growth hormone determinations were performed by N Norman Aker sykehus, Oslo using ^{125}I-labelled hormone (5)

The reliability of the previous intravenous

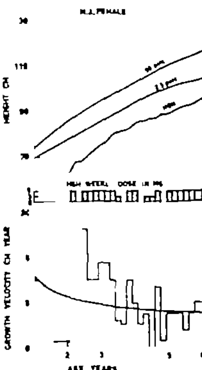


Fig 3 The influence of HGH therapy in Case growth curve and growth velocity

Table 2. *Serum growth hormone levels following insulin induced hypoglycemia*

		Fasting levels		After insulin, 0.1 I u./kg	
Case	Year	Blood sugar (mg/100 ml)	HGH (µg/ml)	Blood sugar (15 min) (mg/100 ml)	HGH (60 min) (µg/ml)
1	(1965	60	20)		
	1966	72	2 6	38	2.6
2	(1965	53	7)		
	1966	53	< 2	-3	< 2

Metopirone test was considered to be uncertain. Oral Metopirone test in 1965 revealed normal response in both patients.

Skeletal age in the boy at the chronological age of 7 $^1/_2$ years corresponded to 6 years. The girl still had normal skeletal age at the age of 6 years. No HGH antibodies have so far been demonstrated in the plasma of the siblings (N Norman, Aker sykehus, Oslo)

COMMENTS

Two siblings with growth retardation since the first months of life are presented. Their father died from diabetes mellitus. Diabetes has also been observed in the family of two other siblings with pituitary dwarfism in the Department of Pediatrics, University of Oslo, Rikshospitalet (6).

In the girl retardation of growth started shortly after birth. Her increase in length during the first four months of life was 3 cm. The boy measured 48.5 cm at birth in spite of a birth weight f 4800 g, and he showed a similar early growth retardation. The diagnosis hypopituitary dwarfism was established at the age of 3 and 1 $^1/_2$ years respectively and was confirmed a few months later by the unequivocal response to substitution therapy Increased insulin sensitivity and low plasma growth hormone levels were demonstrated in 1966

Today it is obvious that these siblings should have been subjected to closer studies on their first admission for growth retardation. The results of long-term therapy with HGH have been promising in both our patients. The catch-up growth was t least as good as reported by others (7 8 10), ith an initial growth velocity of 24 cm year The growth velocity during the first year of treatment was higher in the younger child 13.5 compared to 11.5 cm, and might ha e been even better in both patients if therapy had been continuous. Almost no growth was observed in the periods without therapy A reduction in the dosage to 1 mg three times weekly n the spring of 1964 and 1965 resulted in a marked lowering of growth velocity

Growth is now about average for age in both children. The boy is now 17.5 cm below the 2.5 percentile for his age and the girl is 8.5 cm below her percentile. Even considering the fact that therapy was inadequate for about one year in both patients, it seems obvious that therapy was initiated too late in both cases. The employed dosage, . mg three times weekly cor responded initially to about 17 mg/m^2 surface area per week, which is considerably more than the usual dosage (4 7).

The untoward reactions to the first HGH preparation used (Roos HGH) may have been of pyrogenic nature but could also be due to the lipid-mobilizing component of that preparation (9). Mobilization of fat may have a calorogenic effect with increase of BMR and temperature. The HGH preparation supplied by Trygstad had been freed of the lipid mobilizing component (9)

In 1965 Wilber & Odell (11) described a 7 year-old boy with isolated deficiency of growth hormone and hypoglycemia. Fasting hypoglycemia was observed in both the reported siblings. Thyroid function judged by serum PBI and cholesterol as well as *oral* Metopirone test was normal. Gonadotrophic function is difficult to evaluate during the first years of life.

Moderate normochromic, normocytic anemia was observed in both the reported cases before HGH therapy was initiated A temporary drop in hemoglobin concentration was observed during the first period of therapy particularly in the youngest child. This drop may however be due to untoward reaction to therapy

Little attention has been paid in the literature to hematologic disturbance in hypopituitary dwar fism, and t is difficult to state anything definite about the incidence of anemia in this disorder It is possible that mild anemia usually accompanies the more severe cases of untreated hypopituitary dwarfism This anemia may be due to erythroid hypoplasia. It may be caused by sec ondary hypothyroidism. Thyroid function seemed,

however to be normal in both the reported siblings. Anemia may perhaps be of differential diagnostic value as anemia probably seldom occurs in cases of primordial dwarfism. The rate of growth is so small in these infants that dietary iron deficiency is unlikely to develop. A point to be aware of in the treatment of pituitary dwarfism is the increased iron requirement during the period of catch-up growth. This rapid growth takes place in a period without any iron-fortification of the diet. It seems therefore logical to use prophylactical iron medication at least during the first year of HGH therapy This was done in both the reported cases, even after a normal hemoglobin concentration had been achieved.

It is concluded that in marked pituitary growth retardation, treatment with HGH should be initiated as early as possible in order to obtain a height near the normal range Delayed growth hormone therapy in the first years of life will probably deprive the child of several cm of height.

SUMMARY

Two siblings with hypoglycemia had from early infancy retardation of growth which seemed to be due to isolated deficiency of somatotropin. The father had diabetes mellitus which may be of etiologic significance. They have been followed birth to the ages of 6 and 7 $^{1}/_{2}$ years respectively Experience with long-term therapy with HGH _ mg three times weekly from the age of 3 / $_{2}$ and years is reported. A purified HGH preparation which is supposed to contain only the somatotropin proper seems to be preferable A reduction of the HGH dosage to 1 mg three times weekly resulted in a marked reduction in height increments, and almost no growth was observed in the short periods without therapy Total growth during a 4-year-period was 29.5 and 3_5 cm respectively However the therapy was probably initiated too late and the height continues to be far below the _5 percentile, particularly in the older child. The importance of an adequate therapy from early infancy in the more severe cases of hypopituitary dwarfism is stressed.

Attention is drawn to the hematological aspect of hypopituitary dwarfism, and the increased iron requirement during the initial period with catch-up growth.

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STUDIES ON ERYTHRO-KINETICS IN INFANCY

XII. *Survival in Adult Recipients of Cord Blood Red Cells Labelled* in Vitro *with Di-isopropyl fluorophosphonate* ($DF^{32}P$)

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The life span of most of the red cells formed during foetal life is shorter than that of the red cells formed during adult life. This general statement is born out by several studies using different techniques of investigation (see e.g. 11 17 18, 20, 31).

Little is known about the mechanism(s) responsible for this difference between the two cell types. Information on how cord blood red cells from full-term infants disappear from the circulation of normal adult individuals may be used to obtain further insight into this problem. Available data based on the ^{51}Cr technique (8, 13 14 27 28) and the haemoglobin-F technique on single red cells (2), (7 15 19 22) and on whole blood (24) (21) are inconclusive and partly conflicting. The present work was therefore undertaken to obtain additional information on this point. Cord blood red cells were labelled *in vitro* with di-isopropyl fluorophosphonate ($DF^{32}P$) according to a method (5) which yielded linear disappearance curves in four normal adult autotransfusions and a rate corresponding to a mean red cell life span of 111 days.

MATERIAL AND METHODS

Cord blood was obtained from 9 healthy infants, all judged to be full-term on the basis of clinical examination and gestational age (280 ± 14 days after the first day of their mothers' last menstrual period). At delivery the umbilical cords of these infants were immediately clamped. A sterile catheter was introduced into the umbilical vein and blood from the umbilical cord and placenta was collected under aseptic conditions in ACD-solution (NIH solution B (18)) in the proportions 4 1 and labelled with $DF^{32}P$ (The Radiochemical Centre, Amersham, England) according to the method described in detail by Bratteby & Wadman (5). Between 15 and 50 ml of red blood cells were labelled and the same amount was later injected. $DF^{32}P$ was added to a relative concentration of 0.2 micrograms per ml of packed red cells. No visible haemolysis of the red cells was observed during the labelling procedure. The labelled cells from each infant were injected into a suitable adult recipient. The amount of $DF^{32}P$ injected varied between 0.75 and 2.4 µCi. This will give an absorbed dose of about 100–300 mrads to the blood (9). The absorbed dose to the whole body (= the gonadal dose) will be at the most one tenth of this magnitude.

Blood samples were withdrawn at frequent intervals and the blood was prepared and counted by methods described by Garby (9) and modified by Wadman (29). The statistical counting error was 6–15% in the samples taken towards the end of the study (68–70 days after injection).

The data, expressed as net counts per minute per mg of haemoglobin, were plotted on normal graph paper and the value at day zero was obtained by extrapolation by eye. The extrapolation was performed by omitting the value obtained on the day of injection. Correction factors for sampling were calculated by assuming that all sampled blood was replaced by increased red cell formation during the period up to the next sampling. On this assumption, the maximal correction factor is obtained. This factor was found to be less than 6% of the values obtained at the end of the experiment. As the erythropoietic compensation for sampling is unlikely to be complete the true factor for correction of the effect of sampling is most probably smaller than 6%. Because of the uncertainty in this estimate, no correction for sampling was made. The error of neglecting the sampling effect is small in comparison with the error of determining the relative radioactivity in the samples.

Table 1 *The blood groups (ABO Rh and Kell) of the donor infants, their mothers and the adult recipients*

Mother	A Rh+	A Rh+	A Rh+	O Rh+	O Rh−	O Rh−
Infant	A Rh+ K−	A Rh+ K−	O Rh+ K−	O Rh+ K−	O Rh+ K−	O Rh− K−
Recipient (cardiosclerotic patients)	A Rh+ K−	A Rh+ K−	O Rh+ K−	A Rh+ K−	AB Rh+ K−	O Rh− K−
Symbols in Fig. 1	△	▽	+	○	○	Not included in Fig. 1
Mother	O Rh+	A Rh−	A Rh+			
Infant	A Rh+ K−	O Rh+ K−	A Rh− K−			
Recipient (healthy doctors)	A Rh+ K−	AB Rh+ K−	A Rh+ K−			
Symbols in Fig. 1	□	●				

No jaundice was seen in this infant during the first week of life.

Serological investigations

By means of ordinary blood group serological tests (agglutination test with papain-treated and ficin-treated red cells and indirect antiglobulin technique) the presence of irregular erythrocyt antibodies in the mothers of the donor infants and the presumptive adult recipients as sought. These tests were negative. All infants gave a negative direct antiglobulin (Coombs') test. The blood groups of the mothers, infants and recipients are shown in Table 1 A compatibility test including saline cross-match at 20°C and an indirect antiglobulin test with the recipient serum and the donors red cells was also performed and found to be negative prior to transfusion

Adult recipients

Si of the adult recipients ere male patients, aged 48–80 years, hospitalized for proven or suspected myocardial infarction, but otherwise healthy At the time of the study all patients were in good general condition. During the period of cord red blood cell survival study in these patients their total haemoglobin mass, as measured by the alveolar CO method (25 26) changed by value ranging from +7 to −10% (mean − %). Three of the adult recipients were healthy male doctors aged 30–32 years. The venous haematocrit of these subjects did not change more than ±0.5 units during the study

RESULTS

The results are seen in Fig 1 The disappearance appears to be curvilinear in all cases with a rate of 1 1 1.5% per day during the first 30 days and 0.7 1 1% per day during the following 30 days. No collapse" curves (1) were seen, but in one of the studies, not included in Fig. 1 or the calculations, the rate of disappearance of the labelled cells was much faster than in the studies shown. The recipient was a male patient with an earlier myocardial infarction. During the time of the study his initial blood carbon monoxide concentration was found to be significantly above the normal range for the laboratory (The initial

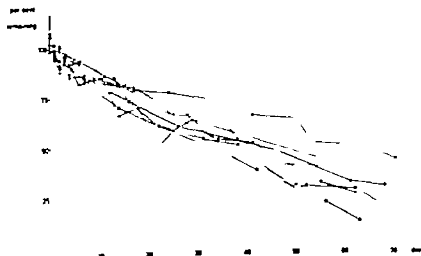


Fig 1 Disappearance of DF³²P labelled cord red blood cells transfused into adult individuals. (The symbols are defined in Table 1)

blood carbon monoxide concentrations in the other cardiosclerotic patients were within the normal range) Other signs of increased haemolysis were not found in this patient.

Some of the variation in the rate of disappearance within and between the different curves is undoubtedly due to experimental error The true variation of disappearance between individuals is therefore less than shown in the figure. However the number of studies is too small to allow any estimate of the variation.

DISCUSSION

Mollison (16) and Seelemann (23) followed the fate of adult red cells in the circulation of newborn and very young infants, with an Ashby technique, and found that these cells disappeared in an approximately linear fashion at a rate of about 1% per day The estimations were approximate because possible changes in the blood volume of the infants during the experimental period could not be evaluated. However the form and rate of disappearance were similar to that found in the adult organism. The results thus indicate that the life span of these cells is almost entirely dependent on "intrinsic" properties of the cells and that possible random events or "extrinsic" factors associated with the final removal of the cells operate only towards the very end of the life span.

Mollison (17 p. 108) transfused cord blood and adult red cells simultaneously into four anaemic premature infants and followed the disappearance rate, using an Ashby technique. He found that the cord blood cells disappeared faster than the adult cells.

Seelemann (23) failed to observe any difference between the disappearance rates for cord blood cells and adult red cells from the circulation in very young infants. The cells were transfused to different individuals, however and the difference noted by Mollison (17) could well have been obscured by different changes in blood volumes among the recipients.

The difference in life span distribution between adult and foetal red cells in the organism of the newborn or very young infants could be entirely due to differences in "intrinsic" properties of the cell types. For example, metabolic processes might deteriorate more rapidly in the foetal cells, thereby shortening the time of their ability. However it is also possible that the difference in life span distribution could be due to external random mechanisms operating upon the foetal cells and not upon the adult cells. The difference in life span distribution would still reflect differences in cellular properties these differences would, however be of a different nature than those mentioned above. For example, external random mechanisms in the circulation of newborn infants could operate more efficiently on cells of larger size.

Accepting that adult cells disappear linearly at a rate of 1% per day in the newborn or very young infant, the cord blood red cells in the studies of Mollison (17) disappeared in a curvilinear fashion at a rate of about 1.2% per day during the first 50 days, and about 0.8% per day during the next 50 days.

If the life span of the foetal cells were determined solely by "intrinsic" cellular properties, the disappearance of such cells from the circulation of adult individuals would be expected to be similar to that found in newborn or very young infants. If, on the other hand, the difference in life span distribution between adult and cord blood red cells in the circulation of newborn or very young infants were dependent only upon differences in the behaviour of "extrinsic" factors towards these two types of cells, the disappearance of cord blood cells from the circulation of normal adults might well be different from that in newborn or very young infants.

Several authors have studied the rate of disappearance of foetal red blood cells from the maternal circulation after foetomaternal transfusions (7 15 21). The results from these studies are not easily interpreted. Two sources of error working in opposite directions, may be mentioned. 1 In the studies mentioned above the authors have assumed that the red cell volume of the recipients is constant during the time of study Caton *et al* (6) have shown, however that the red cell volume decreases gradually by some 40% during the first 60 days of the puerperium. Such a decrease will lead to an underestimation of the disappearance rate of foetal red blood cells from the maternal circulation. 2. The time of occurrence of foetomaternal transfusion is difficult to assess. Cohen *et al* (7) have shown that transplacental passage of foetal red blood cells often takes place during the whole gestation from the end of the first tri-

mester with increasing frequency towards delivery. An apparently high disappearance rate can thus be due to foetomaternal bleeding(s) before delivery.

Pearson (19) using the Betke-Kleihauer technique (?) of detection, found that cord blood pooled from five full-term infants and transfused into a 52-pound child disappeared linearly at a rate of 2% per day during the 27 days of the study. Schneider & Haefele (22) studied the disappearance of cord red blood cells after injection into adult recipients using the Betke-Kleihauer technique (2). The disappearance rate calculated from values of the tables in their article was 6.7% (4.3-8.4%) per day during the first 5-6 days following injection, 2.1% (1.7-2.3%) per day during the rest of the first month, and 2.5% (1.3-3.0%) per day during the second month following the injection. Zipursky (31), in a review article, reported results from unpublished studies in which he used the same technique as Pearson (19) and Schneider & Haefele (22) and found the "life span" of cord blood red cells transfused into normal adults to be between 56 and 105 days in five cases. It is not clear from Zipursky's report what he means by "life span" but from the discussion it appears that the data are actually the reciprocal the (linear?) disappearance rate. The results these three studies (19, 22, 31) using the same technique thus vary considerably.

The data obtained in the present study are in close agreement with those presented by Mollison (17, p. 108). It is therefore most likely that foetal red cells disappear from the circulation of adult, as well as newborn, individuals mainly as a result of their "intrinsic" properties. If extrinsic factors are operating, they are of about the same intensity in the two types of organisms. In any case, the survival of cord blood red cells in the normal adult organism appears to reflect fairly accurately the survival of cord blood red cells in the newborn infant.

The DF^{32}P curves and the Ashby curves are strictly comparable only if it is assumed that there is random labelling of the cord blood red cells by the DF^{32}P. A prerequisite of this assumption is that the number of DF^{32}P molecules per cell is independent of cell age. Furthermore, it is assumed that there is no physical elution of the label from the cells. Both assumptions appear to hold true for the cells of normal adult individuals (5).

The present data give directly the rate of destruction, at any time during the first two months of life, of the red cells present at birth. These estimates are in excellent agreement with estimates of red cell destruction obtained by an analysis of the combined data obtained by independent methods (3, 10, 11, 30). This has been discussed in a previous paper (3) and will be analyzed further in a separate communication (12).

There are many obvious differences between foetal and adult red cells. One type of difference, to the elucidation of which the present data may contribute, is the difference in the rate of *in vivo* elution of ^{51}Cr obtained after standard procedures of labelling. Foconi & Sjölin (8) compared the disappearance of ^{51}Cr-labelled cord blood red cells from the circulation in normal adults, with the data of Mollison (17) and Seelemann (23) on the rate of disappearance of serologically labelled cord blood red cells from the circulation of newborn and very young infants. They concluded that the ^{51}Cr elution from foetal cells is considerably faster than that from adult red cells. Pearson (19), on the other hand, estimated the ^{51}Cr elution from the blood transfused in the experiment referred to above, and found a figure of only 0.8% per day or very similar to that found for adult cells. The present data show a slower disappearance rate of cord red blood cells than that found by Pearson (19) and thus rather support the conclusions of Foconi & Sjölin (8) with respect to the ^{51}Cr elution.

The mean life span and the distribution of life spans around the mean value of the foetal cells cannot be evaluated on the basis of survival studies alone. As pointed out by Mollison (17, 18), placental blood must contain a disproportionate number of young cells. A disappearance rate of 1.0 to 1.5% during the first month after labelling of placental red cells indicates, therefore, that the mean cell life span must be considerably less than 1.0 days. The problem of evaluating the mean life span and life span frequency function will be dealt with in a further communication (4).

SUMMARY

Cord blood red cells from 8 full-term infants were labelled *in vitro* with di-iso-propylfluorophosphonate (DF^{32}P) and transfused into 8 adult recipients.

The disappearance of the labelled cells was slightly curvilinear with a rate of 1.0–1.5% per day during the first month after transfusion and 0.7–1.0% per day during the following month.

The data are discussed with reference to earlier studies and it is concluded that cord blood red cells from full-term infants disappear from the circulation of adult recipients in much the same way as from the circulation of newborn infants. The finding favours the hypothesis that "intrinsic" rather than "extrinsic" factors are likely to cause the difference in life span between foetal and adult red cells.

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STUDIES ON ERYTHRO-KINETICS IN INFANCY

XIII. The mean Life Span and the Life Span Frequency Function of Red Blood Cells Formed during Foetal Life

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The life span of many of the red blood cells formed during foetal life is shorter than that of red cells formed during adult life (cf. 10, 13, 14, 22). However, neither the mean life span nor the life span frequency function of these cells has so far been estimated.

The life span frequency function of red cells formed during stationary conditions, i.e. when the production and destruction of cells have been constant and equal for at least the life span of the longest living cell, is obtained uniquely and numerically fairly simply from an analysis of the disappearance function of randomly labelled cells (cf. 1, 7). In non-stationary conditions, on the other hand, the life span frequency function can be obtained from the disappearance function only if the details of the changes in production and destruction are known (2, 11).

The red blood cells present at birth have been formed during the latter part of foetal life. During this period, neither the rate of production nor the rate of destruction of red cells can be assumed to be constant. Presumably both rates increase. Therefore, the disappearance function of labelled cord blood cells cannot alone give more than a qualitative description of the life span frequency function.

In this communication, we present results of an analysis of the life span frequency function of red cells formed during the latter part of foetal life and present at birth in normal full-term infants. The study is based on two sets of experimental observations: 1) the increase of circulating red cell volume during the latter part of foetal life and 2) the disappearance of randomly labelled cord blood cells from the circulation of normal adults. The method of analysing these two sets of data in terms of the life span frequency function is one of curve fitting and parameter estimation. The numerical computation of parameter values in the present case was found to be quite complex. A computer program was therefore designed to solve this problem (11).

In addition to information about the life span frequency function of the cells formed during the latter stage of foetal life, the study also produced estimations of the production and the destruction of cells during this time period.

THEORY

Within the stochastic frame of reference, the most fundamental property of the red cell system is given by the death probability function $\mu(a)$ of the erythrocytes. This function is defined by Bergner (1) such that $\mu(a) \cdot dt$ is the probability that a cell of age a will die within the interval dt at time t. In the stationary state, $\mu(a)$ is related to the life span frequency function $\varphi(a)$ by

$$\varphi(a) = \mu(a) \exp\left\{ -\int_0^a \mu(x)\, dx \right\} \qquad (I)$$

where

$$\int_0^\infty \varphi(a)\, da = 1 \qquad (II)$$

$\varphi(a)$ is the fraction of cells dying per unit age at age a.

In non-stationary states, the relation between $\mu(a, t)$ and $\varphi(a, t)$ is much more complicated (2) and, accord-

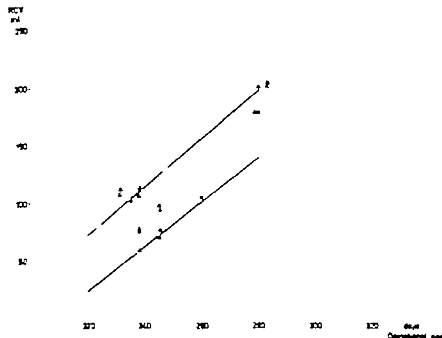


Fig. 1. The relation between the circulating red cell volume and gestational age. Data from □ Brody & Nilsson (5), △ Usher, Shephard & Lind (19) and Usher & Lind (20) and ● Bratteby (3). The range taken as representative for the present calculations is shown.

ingly the lif span frequency function cannot be easily derived from simple assumptions about the mechanisms of cell death. The analysis was therefore restricted to the general form of the life span frequency function and the mean cell life span. As ill be shown in the follow ing, this restriction is ell motivated from the fact that the a ailable experimental data did not allow precise etermination of $\eta(a, t)$. In fact, several different forms of $\eta(a, t)$, i.e. indirectly several different mechanisms for the cell death, were found to be compatible ith the experimental data.

From (II) w find that the relative number of cells surviving at age a, and time t $G(a, t)$, is

$$G(a, \;) = 1 - \int^{a} \;(x, \quad a)\,dx. \qquad \text{(III)}$$

The number of cells of age circulating at time is then equal to $G(a, t)$ multiplied by the number of cells produced per unit of time at time $t-a$, $p(t-a)$:

$$N(a, t) = p(t \quad)\left\{1 - \int^{a} \eta(x, \quad)\,dx\right\} \qquad \text{(IV)}$$

The number of cells of all ges circulating at time t is then

$$N(t) = \int^{\infty} p(\quad)\left\{1 \quad \int^{a} \;(x, t \quad)\,dx\right\} da \qquad \text{(V)}$$

When the circulating cells are labelled randomly i.e. the relative amount of label per cell is independent of cell age, their relative number in the circulation, $N^*(\;)$, is given by (V) by putting $p = 0$ from the time of labelling.

The time dependency of the lif span frequency will be discussed later.

EXPERIMENTAL DATA

The total volume of circulating red cells, $N(t)$, during the time period of 220–280 days f gestation as studied by Brody & Nilsson (5), Usher *et al.* (19), Usher & Lind (20) and Bratteby (3). Since there is little or no change in the mean cell volume during this time period (18), these data can be taken t be representative of the total number of cells in the circulation. Fig. 1 shows the data from the publication of Bratteby (3) together ith the range of values taken t be representative for the present calculations.

The disappearance function of randomly labelled cord blood red cells, $N^*(t)$, has been studied by several investigators. The data have been discussed in some detail by *Bratteby et al.* (4). *Fig. 2 show the experimental data* from the study of these workers and the range of values taken t be representative for the present calculations.

GENERAL METHOD OF COMPUTATION

The problem f estimating the t functions $p(t)$ and $\eta(a)$ from the experimental dat $N(t)$ and $N(\;)$ as approached in the following way (11). Descriptive forms of the functions were chosen on the basis of previous general knowledge (see below). These functions th their (unknow) parameter values ere inserted into equation (V). Parameter values were given (ith certain restrictions mentioned below) by random-number generator nd the values of $N(t)$ and $N^*(\;)$ ere calculated on CD 1600 computer. A sufficient number of curves for $N(\;)$ and $N(t)$ were generated to give approximately 100 curves ithin the experimental range. The parameter alues giv ing compatible curves, e. curves ithin the experimental ange, were thus lly found.

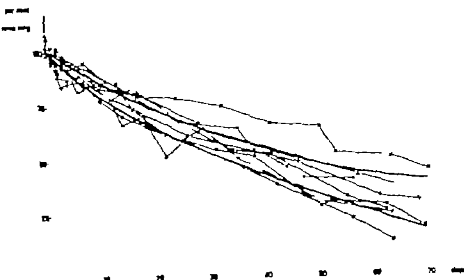


Fig. 2. The disappearance of DFP³²-labelled cord red blood cells from the circulation of normal adults (Bratteby, Garby & Wadman (4)). The range taken as representative for the present calculations is shown.

The functions $p(t)$ and $\psi(a, t)$

The function $p(t)$ was assumed to increase smoothly during the 60 days before birth. The function $p(t) = A + A \tanh(A_2(t-280))$ was chosen to represent this increase, where A and A_2 are the model parameters to be determined. The following restrictions were imposed:

1. $p(t) = 0$ for $t > 280$ days, since only cells formed before birth were studied.
2. $1.5\% < p(280)/N(280) < 3.5\%$ per day. This is a restriction due to the knowledge that the production of red cells at the time of birth is between 2.4 and 3.0% per day (16) or 1.5 and 3.0% per day (9) of the total circulating mass.
3. $0 < p(220) < 5$ ml per day.

The life span frequency function $\psi(a, t)$ was chosen on the basis of the following considerations. During stationary conditions, $\psi(a)$ is uniquely determined by the death probability function $\mu(a)$ (cf. eq. I). Although this relation cannot be expected to hold during non-stationary states in general, forms of the life span frequency function are most conveniently obtained from simple assumptions concerning the death probability function in the stationary state. In the case investigated here, the use of eq. (I) was found to be justified (11).

A $\mu(a)$-function which implies an age independent death probability (death by random events) starting at age A_4 has the life span frequency function

$$\eta_A(a) = A_3 \exp(-A_3(a - A_4)).$$

A $\mu(a)$-function which implies that the age at which the random factors start to operate is different among different cells, has the life span frequency function

$$\eta_B(a) = A\,^2(a - A_4)\exp(-A_5(- A\ )).$$

A $\mu(a)$-function which implies that the death probability increases linearly with age, has the life span frequency function

$$\eta_C(a) = A_5(\ - A_4)\exp(-A_5(\ - A_4)^2/2).$$

These forms of $\psi(a)$, especially their type of skewness, are in agreement with the combined semiquantitative data of Garby *et al.* (10), Wranne (21) and Mollison (13).

The Gaussian form for $\psi(a)$, which has a complicated $\mu(\ )$ function, was also investigated.

All life span frequency functions are normalized according to (II).

The mean cell life span, $T(\ )$, was taken to be equal to the expectation value:

$$\int^{\infty} a\eta(a, t)\,da.$$

The time dependency of $\psi(a, t)$ was studied by using $\eta(a)$ and letting first A and second A vary linearly between 220 and 280 days.

RESULTS

When the value of A in $\eta(a)$ was allowed to increase or decrease linearly between days 220 and 280 within the limits of 1 and 3% per day 155 compatible runs, i.e. the number of curves falling within the two experimental ranges simultaneously were obtained. The results are shown in Fig. 3. The distribution of compatible solutions clustered around the values 1.6 and 2.0% for A both at

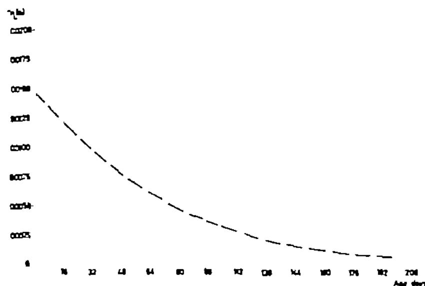
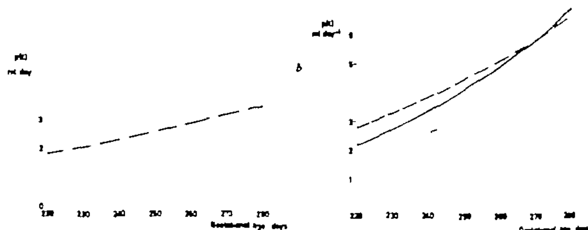
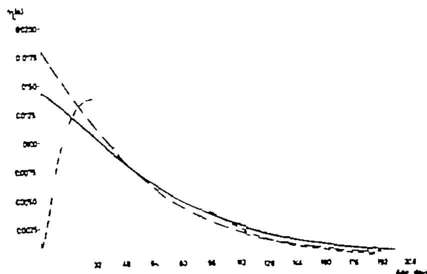


Fig 6. Compatible life span frequency functions, $\eta_B(a)$ and the corresponding curves for red blood cell production, $p(\,)$

(*a*) Function giving maximum (——) value of mean life span ($\bar{T}$).

(*b*) The corresponding function $p(t)$





(*c*) Functions with maximum (——) and minimum ( ) red blood cell production at day 220 of gestation, $p(220)$ and maximum (——) and minimum ( ) production at day 280, $p(280)$.

(*d*) The corresponding functions $\eta_B(a)$.

The values for $\bar{T}$, $p(220)$ and $p(280)$ are given in Table 1

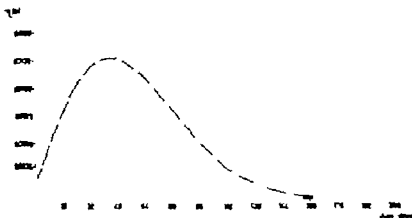
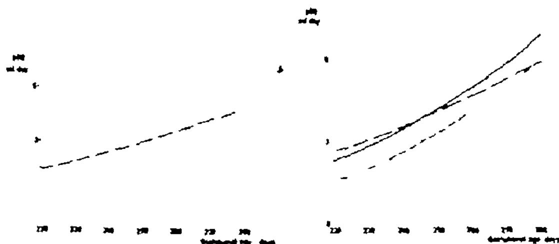
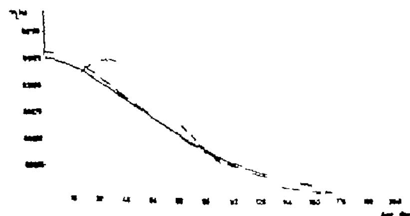


Fig. 7. Compatible life span frequency functions, $\eta_C(\ )$, and the corresponding curves for red blood cell production, $p(\ )$.

(*a*) Function giving maximum (— —) value of mean life span ($\bar{T}$).

(*b*) The corresponding function $p(\ )$.





d

(*c*) Functions with maximum (— —) and minimum (—) red blood cell production at day 220 of gestation, $p(220)$, and maximum (——) and minimum ( ) production at day 280, $p(280)$.

(*d*) The corresponding functions $\eta_C(a)$.

The values for $\bar{T}$, $p(220)$ and $p(280)$ are given in Table 1.

Table 1 *The mean cell life span* $\bar{T}$ *(days) and the production of red cells (ml/day) at day 220* p (220), *and day 280* p (280) *of gestation from selected compatible runs of the three different* η*-(a)-functions*

Mode of selection	$\eta_A(a)$			$\eta_B(a)$			$\eta_C(a)$		
	$\bar{T}$	$p(220)$	$p(280)$	$\bar{T}$	$p(220)$	$p(280)$	$\bar{T}$	$p(220)$	$p(280)$
Maximum $\bar{T}$	71	1.5	4.8	61	1.9	4.2	57	1.8	3.5
Mean $\bar{T}$	58	2.0	5.0						
Minimum $\bar{T}$	43	2.5	4.7	43[a]			43[a]		
Minimum p at $t = 220$	63	1.5	5.9	55	1.6	5.4	52	1.6	5.5
Maximum p at $t = 220$	55	2.6	5.8	49	2.8	6.6	52	2.6	6.3
Minimum p at $t = 280$	67	1.9	3.6	59	2.1	3.9	57	1.8	3.5
Maximum p at $t = 280$	62	2.3	6.7	58	2.2	6.8	55	2.0	6.7

[a] Minimum and mean values of $\bar{T}$ for $\eta_B(a)$ and $\eta_C(a)$ approach that for $\eta_A(a)$.

DISCUSSION

The results of the present study show that the life span frequency function, $\eta(a)$ is fairly constant during the latter part of foetal life and that the mean life span of the cells formed during that period is between 45 and 70 days. Furthermore, the distribution of life spans around the mean value is quite large, i.e. many cells die before reaching an age of 20–30 days and many cells live up to the age of 100–140 days. The conclusion that a considerable fraction of the cells have a very short life span is in agreement with previously published findings (10), and the presence of cells living for 110–130 days has been proven by Hosoi *et al.* (12) and others.

It is highly unlikely that different forms of the function $\eta(a)$ would have given materially different results, but it is not possible to prove this statement rigorously.

As shown by Mollison (13) and Seelemann (15) (cf. 4), there appears to be little difference in the rate of disappearance of cord blood cells and adult red cells after transfusion into very young infants. Also, adult red cells are recovered from the circulation of very young infants in much the same way as they are removed from the circulation of adults. Furthermore, Bratteby *et al.* (4) showed that cord blood cells disappear from the circulation of normal adults in much the same way as they disappear from the circulation of very young infants. These findings make it unlikely that "extrinsic" random factors determine the life span of the red cells in very young infants. Rather the data favour the hypothesis that "intrinsic" factors in the foetal cells determine their life span. If this hypothesis is accepted, the present data must be interpreted to mean that these "intrinsic" factors are distributed very differently among the cells formed during the period in question. Such a wide distribution of intrinsic factors could arise from differences in the cells when they are formed. The differences might also arise from a varying degree of external influence in the foetal organism resulting in a wide distribution of different rates of ageing of the red blood cells.

The morphological and biochemical counterparts of the "intrinsic" factors are not defined in the present study. However, it is of considerable interest to note that the macrocytic erythrocytes produced in rats and rabbits in response to large doses of erythropoietin (17) and to low barometric pressures (8) also survive for only short periods. Card & Valberg (6) reviewed the data on the survival of "stress" erythrocytes and showed that the macrocytic red cells produced by administration of phenylhydrazine in rabbits not only have a very short mean life span but also that some of the cells survive as long as normal cells. The erythrocytes produced during foetal life have a larger mean cell volume than those produced later in life. Also, they are produced at a much more rapid relative rate (cf. Garby *et al.* (9), and the present work). In these respects, the cells are similar to the "stress" cells discussed above. The fact, shown here, that the foetal cells also have the same survival characteristics makes the analogy fairly complete (cf. Zipursky (...)).

The life span frequency function was found to be essentially independent of time during the time period investigated. A possible corollary to this finding may be the fact that there is very little change in the mean cell volume during the last two months of gestation (18).

There are no published estimates of the production and destruction of red blood cells during foetal life. The present data offer approximate estimations and indicate that the rate of production per unit of red cell mass is of the order of 3–5 times that found in normal adults. This estimate is in agreement with the fact that the reticulocyte concentration at the time of birth in premature infants is larger by a similar factor than that found in adults (16). The rate of destruction of erythrocytes appears to increase quite considerably towards the end of the gestation period. The wide range in these two estimates probably does not reflect a true biological variation. Rather the large variation is probably due to the wide range in the estimate of the red cell mass as a function of gestational age, the uncertainty of this estimate is most probably due to the unreliability of the determination of the gestational age.

SUMMARY

Equations were developed for the relation between the production rate, the life span frequency function and the number of circulating erythrocytes and for the relation between the production rate, the life span frequency function and the relative number of circulating cells after random labelling.

With the help of explicit expressions for the production rate and the life span frequency function of erythrocytes formed during the latter part of foetal life the increase in number of circulating cells during this period and the relative decrease in circulating labelled cells in a simulated cord red blood cell survival experiment were calculated on a digital computer and compared with the experimental data. Through the simulation procedure, the parameters in the life span frequency function and the production rate function were estimated.

The life span frequency function of the red blood cells did not change appreciably during the last 60 days of foetal life.

The mean cell life span was found to be between 45 and 70 days. The life span frequency function was skew with the majority of cells dying before the mean life span.

The relative rate of production of erythrocytes during the last two months of gestation was found to be 3–5 times that of normal adults.

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ENDOGENOUS FORMATION OF CARBON MONOXIDE IN NEWBORN INFANTS

IV On the Relation between the Blood Carboxyhaemoglobin Concentration and the Pulmonary Elimination of Carbon Monoxide

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Increased concentrations of carboxyhaemoglobin (COHb) in blood have been found in newborn infants with haemolytic diseases (9 10), and with so called physiological jaundice (2). The COHb level in blood mainly depends on the endogenous formation of CO from degrading haemoglobin (5 16–19), but other factors, such as the alveolar ventilation, may influence the level (4 16).

Since disturbances of the pulmonary function, not clinically recognizable as respiratory distress, may be common during the first days of life, it would be of interest to study the relationship between the COHb level and the CO elimination (V_{CO}) in newborn infants. This would allow an evaluation of the significance of the increased COHb concentrations. With that aim in mind a method for the determination of the V_{CO} in small infants was developed, and the above mentioned relationship was studied in different groups of full-term newborn infants.

MATERIAL

The material consisted of 43 newborn infants, on whom simultaneous determinations of the COHb concentration and the CO elimination were done. All infants were in good general condition, and none displayed any clinical signs of respiratory disease.

Since preliminary investigations had shown that infants breathing room air with comparatively high CO concentration added practically no CO to the expired air it was decided in advance only to include investigations with room air CO below 1.5 parts per million (ppm).

The 43 infants comprised 15 full-term infants without known haemolytic disease, 10 infants with jaundice and ABO incompatibility and 18 infants with Rh haemolytic disease

The 15 full-term infants without known haemolytic disease (Nos. 1 15) had red cells compatible with the maternal blood with respect to the Rh factor and the ABO blood groups. The direct antiglobulin reaction was negative in all infants. The haemoglobin concentration and number of reticulocytes were normal The bilirubin concentration at the time of investigation ranged from 0.7 to 32.9 mg per 100 ml, exceeding 10 mg per 100 ml in eight infants (Nos. 8–15). One infant was asphyctic at birth but recovered within a few minutes (No. 15). One infant was delivered by means of vacuum extractor (No. 11).

The 10 infants with jaundice and ABO incompatibility (Nos. 16–25) were full-term and had bilirubin concentrations ranging from 9.0 to 27.5 mg per 100 ml. Rhesus isoimmunization was excluded in all cases.

In both these groups all mothers were non-smokers No infant was studied after exchange transfusion

Eight of the infants with Rh haemolytic disease were studied on the first day of life (Nos. 26–33). All these infants displayed unequivocal signs of increased haemolysis necessitating exchange transfusion according to our criteria (9). Three of the mothers were smokers (Nos. 26–28). One infant was investigated after exchange transfusion (No. 29). No volatile anaesthetic, except nitrous oxide, was used at the delivery in this group.

Ten other infants with Rh haemolytic disease were studied after the first day of life (Nos. 34–43). The haemolytic disease was less severe than in the previous group, and in only two of the infants exchange transfusion had to be done on the first day (Nos. 37 and 38). Three of the mothers were smokers (Nos. 34–36)

Although delivery had been induced prematurely in several cases, the birth weight exceeded 2500 g in all the infants with Rh haemolytic disease

METHODS

Determination of the pulmonary elimination of carbon monoxide (V_{CO})

General principle of the method An open circuit

Fig 1 ( ) The breathing mask (*b*) infant attached t the mask.

system as used with the infant attached by means of nose mask without valves. The infant breathed room air which was sucked through the nose mask at constant flow rate and collected in bags. The pulmonary elimination of CO per unit time as determined from the increase of the CO concentration in the air during the passage and the olume of air passing through the mask. A Hopcalite CO meter was used for the CO analyses. Since the amount of CO eliminated per unit time as small (0 5 6 μl per min), and the xpired air was diluted during collection, pecial precautions were necessary t obtain known, constant CO concentration in

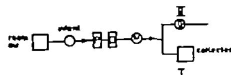


Fig 2 The system for collection of expired air P Filter with potassium hydroxide· D, filter with soda lime (Durasorb)· M, membrane pump; G, gas meter

the inspired air and to prevent contamination of the collected air with exogenous CO or ith other volatile substances combustible in the CO meter Furthermore, a high degree of accuracy for the CO analyses was necessary

The breathing mask. A breathing mask of the same design as that described by Geubelle *et al.* (17) as used (Fig 1) It consisted of tw short (5–8 mm) pieces of latex tubing, the diameter of which was chosen to fit well into the infant nares, connected to piece of larger tubing (length 15–20 cm, inner diameter 7 mm). As practically all healthy quiet newborns prefer breathing through the nose, they inspire from and expire to the air passing through the larger tubing Leakage at the side of the mask as prevented by covering the pas between the larger tubing and the tip of the nose ith anaesthetic jelly I order to prevent leakage through the mouth, it as covered with piece of plaster hich, however did not prevent the infant from opening his mouth acti ly

The open circuit breathing system (Fig). Room air was sucked through the mask at flow rate of 1 45 0 13 l min STPD (mean alue and standard deviation

Table 1. *Results of investigations into the possible sources of systematic errors in the method for* $\dot{V}_{CO}$ *determination*

		n	I		II		Difference (I–II)		*t*-value (*t*-test for paired samples)
			Mean	S.D.	Mean	S.D.	Mean	S.D.	
The breathing system: CO conc. (ppm) in air before (I) and after (II) passing the system with no infant attached		18	1.00	0.44	0.99	0.38	0.006	0.10	0.249
The plastic bags: Initial (I) and final (II) conc. of CO (ppm) and O_2 (per cent) in gas mixture kept in polyvinylchlorid bags for 24 hours	CO (low):	11	1.56	0.93	1.57	0.95	−0.008	0.09	0.302
	CO (high):	8	664	25	665	25	−0.88	8.1	0.306
	O_2	4	52.73	13.73	52.75	13.81	−0.0125	0.404	0.062
The flow rate (l per 10 min): Measured before (I) and after (II) air collection		40	14.6	1.29	14.5	1.33	0.098	0.40	1.550
The CO elimination (µl per 10 min): During the first (I) and second (II) period of air collection		40	12.5	9.3	12.7	9.8	−0.21	3.64	0.370

for all investigations). This flow rate was found to be an acceptable compromise between two different requirements. Ideally the lowest flow rate not giving rebreathing should be used. On six occasions with full term newborns attached to the system, the CO_2 concentration in the efferent part of the breathing mask was measured continuously at different flow rates with a Beckman CO analyzer Model LB-1. Judged from the increasing CO concentration, slight rebreathing was found at a flow rate of 1.0 l/min but not at 1.3 l/min. With the above mentioned flow rates rebreathing could be assumed to be slight, if not excluded altogether. The volume of air passing through the mask per minute was about three times the respiratory minute volume found in normal full term newborns (6, 8), causing a dilution of the infant's expired air. This dilution should be kept as small as possible (see below).

From the mask the air was sucked through one filter with potassium hydroxide pumice and one filter with soda lime (Durasorb[2]) (Fig. 2). It then passed a membrane pump[3] and thereafter one of two alternative ways. Either the air was collected in plastic bags through connection I (Fig. 2), or the flow rate was measured by means of a gas meter type D 10/U [illegible] (connection II, Fig. 2). With this arrangement no component of the system used at the collection of expired air added or absorbed significant amounts of CO. This was tested in separate investigations by analysing the CO concentration in air before and after it had passed the system with no infant attached (Table 1). The gas meter however could not be included in the system during collection of the sample, since it was found that it added considerable amounts of CO or another gas combustible in the CO meter to the passing air.

The gas collection. The air was collected in bags made of polyvinylchloride plastic, which were washed twice with "CO-free" air from the CO-meter (see below) before use. Certain precautions had to be made to avoid contamination of the collected sample. Collected in some bags, CO free air caused only small, irregular deflections of a few mm height when analysed in the CO meter, but in other bags it caused conspicuous deflections, sometimes amounting to 30–40 mm. Apparently in these bags a combustible gas was added to the air. All bags were regularly tested with CO free air and only those with the first mentioned qualities were used. The analyses were always done within a few hours after collection of the sample. The CO and O_2 concentrations in air samples kept in the bags for 24 hours, did not change (Table 1).

Determination of low concentrations of CO in air. Low CO concentrations in air were determined according to Linderholm & Sjöstrand (13) using a Stålex CO-meter type SL[5] set on maximum sensitivity. The output from the CO-meter was registered on a Philips potentiometer recorder type PR 2210 U with full scale deflection for 1 mV and chart speed 160 mm/hour. Between analyses, room air was continuously sucked through the CO-meter by a Reciprotor pump type 404 CP[6] (Fig. 3) with a constant flow rate of about 1.4 l per min. Before entering the CO-meter the air passed through two filters with

[1] MIE Ltd, Manchester.
[2] Lindfors El, Solna, Sweden.
[3] AB Nordgas, Stockholm, Sweden.
[5] AB Stålex, Stockholm.
[6] Reciprotor A/S, Copenhagen-Bagsvaerd.

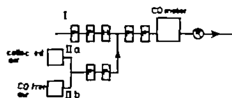


Fig 3 Principle arrangement of the air flow through the CO meter I, Continuous flow of room air between analyses; II, flow at analysis. P potassium hydroxide· H Hopcalite· R, reciprotor pump.

potassium hydroxide purum, one filter with Hopcalite and two other filters with potassium hydroxide (connection I, Fig. 3), removing all CO and drying the air. This arrangement gave a stable baseline on the potentiometer writer with noise level of about 1 mm (Fig. 4). During the analysis the room air was replaced by the sample, passing through four filters with potassium hydroxide before reaching the CO-meter (connection II *a*, Fig. 3). A period of three minutes was chosen for the analysis. Thereafter the tubings and the filters in connection II were flushed with CO-free air (connection II *b*, Fig. 3) for t enty seconds, time estimated to be appropriate to wash out the sample a in this part of the system. The CO-meter was then again run with room air through connection I. Each sample was analysed in duplicate, and before and after the tw analyses a calibrating gas with know concentration of CO in air was analysed (Fig 4).

In the Stalex CO-meter CO is oxidized in the presence of the catalyst Hopcalite and the liberated heat measured with thermistors incorporated in Wheatstone bridge. The height of the deflection of the potentiometer writer caused by the output from the CO meter has shown to be proportional to the CO concentration the sample (13).

Calibrating gases were regularly analysed against dilutions of "pure (99 7) CO" in CO-free air. The procedure was essentially the same as that described by Bjure (1). Small amounts of "pure CO were measured in micro yringe and diluted in 100 l of CO-free air obtained from the CO-meter. During the first part of the investigation the calibrating gas contained 1.93 ppm CO (mean alue of 22 analyses against dilutions, with standard deviation 0 095 ppm). Later calibrating gas containing ..34 ppm CO was used. This gas was analysed 13 times against dilutions (mean alue 34 and standard deviation 0.107 ppm CO) and 15 times against the first mentioned calibrating gas (mean alue 34 and standard deviation 0.113 ppm CO).

The CO concentration of the sample was calculated from the height of the deflection for the sample and for the calibrating gas, and the CO concentration of the calibrating gas. The random error of single determination, calculated from duplicate analyses, was found to be ±0 07 ppm, or 5 4 per cent of the mean (Table 2).

Drägerwerk, Lübeck.
Manufactured by AGA, Stockholm
Matheson Co Inc, East Rutherford, N J
Hamilton Co Inc, Whittier, Calif

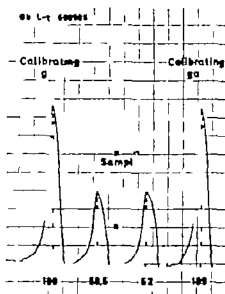


Fig 4 Registration on the potentiometer writer ith duplicate analyses of sample and calibrating gas.

Procedure In order to avoid change of the CO concentration in the inspired air at the beginning of the investigation and to obtain known, constant concentration during the investigation, room air was collected from the ward for 30–60 min prior to the study. The infant as then attached to the nose mask, and the room air from the ward was sucked through the system. During the first 15 min the flow rate through the system was measured (Fig. .., connection II). During the following two 10 minute periods the air was collected for analysis (Fig. .., connection I) and then the flow rate as measured again. I three infants, only one flow rate determination and one air collection was done. No significant difference was found between the first and the second determination of the flow rate (Table 1). The infants ere studied sleeping, as rule about one hour after feeding. Pulse rate, respiratory rate and breathing sounds were checked before the investigation and ith

Table 2. *Random errors of the methods calculated from the standard deviation of the differences between duplicate determinations*

	No. of duplicate determinations	Error of single determination	Error of mean (%)
CO concentration in air (ppm)	76	0 069	5 4
CO increment (ppm)		0 069	8 3
Flow rate (l per 10 min)	40	0 28	2.0
V_{CO} (µl per 10 min)	40	2.6	?0 4

five minutes intervals as long as the infant breathed through the mask. Most infants accepted the mask without displaying any signs of discomfort, and pulse rate and respiratory rate did not change significantly. About one out of ten infants did not accept the mask and the study had to be interrupted.

Calculation and random error of the method. The $\dot{V}_{CO}$ in µl STPD per 10 min was calculated as the product of the volume of air in l STPD passing through the breathing mask during the 10 minute period and the increase of the CO concentration of the air in ppm. No correction was made for the oxygen uptake. The flow rate determination before and after the two periods of air collection was used in the calculation of the $\dot{V}_{CO}$ during the first and second period respectively. No significant difference was found between the $\dot{V}_{CO}$ during the two periods (Table 1). The random error of the method calculated from duplicate determinations was ±2.6 µl CO per 10 min, corresponding to ±20 per cent of the mean (Table 2).

Determination of the COHb concentration. Blood for COHb analysis was drawn immediately before or after the collection of expired air. COHb was determined according to Linderholm *et al.* (14). The random error of a single determination has earlier been calculated to be ±0.05 per cent COHb (9, 10).

The statistical methods used are mentioned in the text. A five per cent significance level was used throughout ($p < 0.05$).

RESULTS

Newborn infants breathing room air with less than 1.5 ppm CO added significant amounts of CO to the expired air (Table 3). In the table are shown the CO concentrations in room air and collected air and the calculated values for the pulmonary CO elimination ($\dot{V}_{CO}$) per unit time, together with the COHb levels and the age and weight of the infants at the time of investigation.

In Fig. 5 and Table 4 the $\dot{V}_{CO}$ per kg and unit time in the different groups can be compared. If the CO elimination in full-term, healthy newborns with bilirubin levels below 10 mg per 100 ml (Nos. 1–7) was used as a basis for comparison, the infants belonging to the other groups were found to eliminate significantly greater amounts of CO (Wilcoxon two-sample-rank test). This still holds true, if the infants possibly influenced by exogenous CO were excluded.

In all groups of newborn infants, included in this investigation, a significant correlation between $\dot{V}_{CO}$ per kg and unit time and the COHb concentration was found (Fig. 6 *a–d*). Multiple covariance analysis according to Brownlee (3) showed that the regression coefficients of the four lines did not differ but that the mean values of the groups differed significantly from a hypothetical common regression line. The regression lines can therefore be regarded as parallel but not as identical. This result implies that at a given

Table 3. *CO concentration in room air and diluted expired air (exp air), calculated mean CO elimination ($\dot{V}_{CO}$) and COHb concentration for each infant together with weight and age at the time of investigation*

Case no.	Age (h)	Weight (kg)	COHb %	Room air CO (ppm)	Mean CO diluted exp. air (ppm)	$\dot{V}_{CO}$ µl per 10 min
1	73	3.32	0.62	0.67	1.24	7.7
2	127	3.34	0.82	0.79	1.15	5.0
3	84	3.65	0.66	0.56	1.16	9.5
4	77	2.80	0.64	0.66	0.99	4.6
5	73	3.44	0.85	0.77	1.30	7.2
6	93	3.17	0.78	0.57	1.06	6.6
7	89	3.90	0.81	0.98	1.29	4.3
8	84	3.48	0.99	0.60	1.38	10.3
9	74	2.72	1.02	0.61	1.44	11.2
10	87	2.96	0.89	1.03	1.56	7.3
11	86	3.86	1.53	0.96	1.92	14.8
12	69	3.19	1.04	0.79	1.64	12.4
13	84	3.34	0.78	0.52	1.02	6.6
14	89	3.45	1.10	1.19	1.61	5.9
15	62	4.00	1.38	0.92	2.14	19.8
16	88	2.92	0.91	0.66	1.16	6.4
17	75	3.80	1.02	0.86	1.78	12.2
18	124	3.78	1.09	0.69	1.17	8.4
19	107	2.98	0.97	0.93	1.52	8.3
20	61	4.11	1.39	0.99	1.89	13.7
21	77	2.81	0.86	0.95	1.09	2.0
22	76	3.08	1.03	0.98	1.41	6.5
23	17	3.70	2.16	0.69	2.33	22.2
24	79	3.48	1.69	0.66	1.79	13.7
25	74	3.08	0.91	0.73	1.30	7.7
26	3	3.42	2.29	1.20	2.69	21.0
27	15	4.48	4.40	0.66	4.21	56.2
28	5	4.55	1.22	0.78	1.93	20.0
29	13	3.78	2.84	0.69	2.87	34.6
30	2	2.51	2.20	0.94	1.62	10.0
31	8	3.68	1.73	0.93	2.42	21.9
32	2½	3.72	1.20	0.89	1.47	9.0
33	8	3.40	1.12	1.06	2.04	11.6
34	74	2.34	1.51	1.15	2.22	14.0
35	69	3.00	1.40	1.00	1.74	12.0
36	33	3.36	0.82	0.72	1.34	9.6
37	90	3.22	1.83	1.25	1.98	10.2
38	64	2.98	1.12	0.77	1.33	8.7
39	73	3.34	1.15	0.81	1.45	10.5
40	28	3.48	0.87	0.78	1.48	10.9
41	70	2.96	1.99	0.78	1.84	15.9
42	162	2.46	0.85	1.11	1.45	5.0
43	62	3.28	1 4	0.89	1.43	9.7

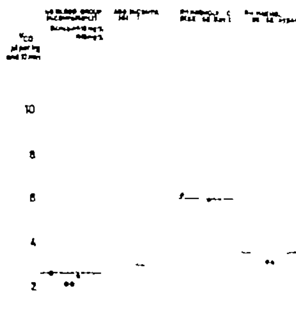


Fig 5 CO elimination (V_{CO}) per kg and unit time in different groups of newborn infants.

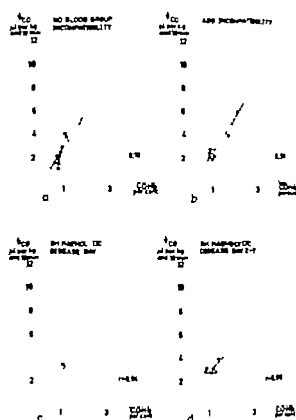


Fig 6 Relationship between CO elimination (V_{CO}) per kg and unit time and the COHb concentration in blood. (*a*) N blood group incompatibility $\hat{y} = 2.98x - 0.15$, $r = 0.68$. (*b*) ABO incompatibility $\hat{y} = 2.99x - 0.70$, $r = 0.39$. (*c*) RH haemolytic disease, day 1 $\hat{y} = 2.85x - 0.05$, $r = 1.58$ (*d*) RH haemolytic disease day 2 7 $\hat{y} = 2.04x$ 0.94, $r = 0.91$

COHb concentration the mean V_{CO} in the groups differed significantly but for a given increment of COHb an equal increase in V_{CO} was found in all four groups. The figure shows that the difference between the groups, although significant, was comparatively small. The vertical distance between the lines was small compared with the random error of the V determination.

In the infants without blood group incompatibility and known haemolytic disease (Nos. 1–15) a significant correlation was found between the V_{CO} per kg and unit time, and the bilirubin level ($\hat{y} = 0.090x + 1.56$ $r = 0.73$)

DISCUSSION

Technical considerations

The pulmonary elimination of carbon monoxide has been studied in adults by Sjöstrand (17) and in small infants by Wranne (20, 21) by the deter

Table 4 *Mean values and standard deviations for CO elimination (V_{CO}) per kg and unit time COHb concentration and venous haemoglobin concentration in the investigated groups of full-term newborn infants*

	N blood group incompatibility						Rh haemolytic disease			
	Bil conc. < 10 mg/100 ml (n 7)		Bil conc. > 10 mg/100 ml (n 8)		ABO incompatibility (n 10)		Day 1 (n 8)		Day 2–7 (n 10)	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
V_{CO} (µl per kg and 10 min)	1 90	0 5	3 24	1 14	2 90	1 40	6.00	3 36	3 56	1.22
COHb	0 74	0 10	1 09	0 25	1 20	0.42	2.13	1 11	1.28	0 41
Haemoglobin (g per 100 ml)	20 6	1 5	20.4	1 8	19 8	3.1	16 1	2.7	16 7	2 4

mination of the respiratory volume and the CO concentration in inspired and expired air. The difficulties encountered in the quantitative collection of expired air in newborn infants have been discussed by several authors (7, 15, 20). The procedure used in the present investigation offered some advantages in this respect.

The dead space of the breathing mask used in the investigation is negligible and the respiratory resistance can be assumed to be low. The simple construction of the mask reduced the risk of leakage. Because of the position of the pump the probable effect of a leak should be an entrance of room air into the system. However, since the CO concentration in air from the ward and from the laboratory usually did not differ considerably minor leakage should not cause a significant error in the V_{CO} determination.

Since the breathing mask did not contain any valves, the air had to be sucked through the system at a flow rate exceeding 1.0 l STPD per min in order to avoid rebreathing. In this way the determination of the respiratory volume was replaced by the determination of the flow rate, which could be accomplished with a random error of ± %. With the flow rate used, an air sample collected during 10 min was sufficient for duplicate analyses in the CO-meter. The procedure however caused a dilution of the expired air with room air, reducing the difference between the two CO concentrations. In this way the percental error in the determination of this difference was increased.

The determination of low CO concentrations in air such as found in room air and expired air was found to necessitate meticulous precautions to avoid contamination with CO from other sources, or with other volatile substances combustible in the CO-meter. For this reason the gas meter could not be included in the system during the collection of expired air. Determination of the flow rate separately i.e. before or after air collection should be satisfactory since the membrane pump ga e a very constant flow. Since the Hopcalite CO-meter is not specific for CO, it is possible that the contamination was caused by combustible constituents in the solvents or lubricants used in the equipment. But it was also found that the degree of contamination increased after that the equipment had been used for gases with higher CO concentrations.

The CO concentrations in room air found during the period of investigation were approxima in the same range as those reported by W n (20) but lower than those given by Coburn al (4). The Hopcalite method probably es mated the room air correctly on most occasions but sometimes the concentration might ha been overestimated because of the lack of pecificit in the method. The combustible gas added to the passing air by the infant, cannot reasonably be assumed to be anything but CO. Using another method for the CO analyses, Wranne found that full-term healthy newborns, with maximum bilirubin concentration below 10 mg per 100 ml and aged 3–6 days on the average eliminated 10.3 µl CO per kg and hour (21). In the present investigation an equivalent group of infants (Nos 1–7) eliminated 11.4 µl CO per kg and hour. The close agreement between the two studies strengthens the reliability of the results.

The random error of a single V_{CO} determination was relatively large. The errors in the determinations of flow rate and CO concentrations should account for approximately half the variation between duplicate determinations of V_{CO}. With a different procedure for the collection of expired air and a different CO method (20) Wranne estimated the pulmonary elimination of CO in newborn infants with the same accuracy (± 0.27 µl per min), as that achieved in the present study (± .6 µl per 10 min). The considerable variation between duplicate determinations, of the same magnitude in both investigations, could speak in favour of an appreciable biological variation.

Biological implications of the esults

From the results of V_{CO} determinations in full term, healthy newborns, Wranne calculated that the daily CO elimination corresponded to the destruction of 1.5 per cent of the total haemoglobin mass and that the total CO-pool in the body equaled 18 hours CO elimination (21). Together with the values for the COHb concentration in healthy newborns reported earlier (2, 9) these figures indicate an efficient CO elimination during the first days of life. The result of the present investigation provides further evidence for an unimpaired CO elimination in these infants, a well as in infants with physiological jaundice or with haemolytic disease of the newborn. In all groups increased COHb levels occurred in con-

nection with increased CO elimination (Fig. 6). If the increased COHb concentrations were the result of CO retention, one would expect to find low V_{CO} values combined with high COHb values. No such combinations were encountered in this study.

The linear regression of COHb upon V_{CO} differed slightly but significantly in the four groups, in that the vertical distance between the regression lines differed from zero, while the slope of the lines did not differ. As several factors may influence the relation between the two variables (4), there can be many explanations for the encountered difference. A considerable variation of the haemoglobin concentration was found both within and between the groups (Table 4), which might explain the difference between the regression lines.

Significant amounts of CO can be supplied to the infant through the placenta by the smoking mother (11), or at exchange transfusion. For this reason exogenous CO most likely contributed to the COHb level in four cases (Nos. 26–29). This fact does not seem to have influenced the V_{CO}/COHb relation, which probably is independent of the origin of the CO. In these infants neither COHb nor V_{CO} can be assumed to reflect haemolysis or haemoglobin catabolism. Exogenous CO should be eliminated within 24 hours (see above) and therefore cannot have influenced the COHb level significantly at the time of investigation in any other infants.

In icteric newborn infants without blood group incompatibility and known haemolytic disease increased COHb concentrations, significantly correlated to the bilirubin concentrations, have been reported earlier (2). It was therefore of interest to demonstrate a significant correlation not only between V_{CO} and COHb but also between V_{CO} and the bilirubin concentration in such infants. These findings provide further evidence for the hypothesis that increased haemoglobin catabolism contributes to the hyperbilirubinaemia in newborns with physiological jaundice.

Markedly increased COHb concentrations have been found in infants with Rh haemolytic disease (9) and in icteric infants with ABO incompatibility (10). It is reasonable to interpret the increased COHb level in these cases as a sign of increased haemolysis, an interpretation supported by the results of the V_{CO} determinations. In moderate to severe cases of Rh haemolytic disease not only increased, but rising COHb levels have been found during the first day of life (9). Since the investigation showed an efficient CO elimination in such infants, their rising COHb level should indicate an increasing rate of haemolysis. The alternative explanation, CO retention, seems unlikely in the light of the present results.

In a steady state with constant CO concentration in the inspired air and constant rate of endogenous CO formation, the pulmonary CO elimination should give quantitative information about the haemoglobin catabolism. The good correlation between V_{CO} and COHb implies, that the COHb concentration in blood gives the same information about haemoglobin catabolism as the pulmonary CO elimination. The COHb can be determined easier and with greater precision than the V_{CO}. Furthermore, there are reasons to believe that the COHb level is less dependent on temporary variations in the pulmonary ventilation and perfusion than the CO elimination (4).

SUMMARY

A procedure for the determination of the pulmonary elimination of carbon monoxide in newborn infants has been described. Quantitative collection of the expired air was accomplished with the aid of an open circuit breathing system. The low CO concentrations in room air and the collected air were measured in a Hopcalite CO-meter.

Simultaneous determinations of the blood carboxyhaemoglobin (COHb) concentration and the pulmonary elimination of carbon monoxide were performed on 15 full-term newborns without blood group incompatibility and known haemolytic disease, on 10 infants with jaundice and ABO incompatibility and on 18 infants with Rh haemolytic disease. In all infants the birth weight exceeded 2500 g and none of the infants displayed any clinical signs of respiratory disease.

In all groups a significant positive correlation was found between the pulmonary CO elimination and the COHb concentration. The results indicate that in full term newborn infants, the pulmonary CO elimination is efficient from the first day of life even in the presence of haemolytic disease and anaemia. The increased COHb concentrations found in these groups of new-

borns can thus not be explained by CO retention but should reflect an increased haemoglobin catabolism.

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ECG COMPARED WITH MYOCARDIAL ULTRASTRUCTURE IN ANOXIC FOETUSES OF NORMAL AND HYPERGLYCAEMIC RABBITS

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We recently reported that the content of glycogen in the heart muscle of rabbit foetuses could be augmented if the mother received a glucose infusion prior to delivery (6) Such an infusion also resulted in prolonged maintenance of foetal heart activity when the foetuses, after delivery were subjected to anoxia. The heart function, as reflected by the ECG deteriorated during anoxia, and it can be anticipated that underlying ultrastructural changes were present in the myocardial cells, but that these changes possibly appear later in foetuses of glucose-infused mothers. In available literature we have not been able to d such an investigation published before. herefore, an account is given in this paper of a comparison between the ECG and the fine heart structure of control foetuses of untreated rabbits and of foetuses of glucose-infused mother rabbits.

MATERIAL AND METHODS

The technique of studying concurrently the foetuses of tw rabbits, one control and one glucose-infused animal, on the 29th day of pregnancy was that previously de scribed (6). The present material for electron microscopy was obtained from two such pairs of rabbits. The controls weighed 3300 g (C_1) and 3600 g (C_2), and the glucose-infused rabbits 3400 g (G_1) and 4000 g (G_2) In each of the experimental animals, 120 ml of 30% glucose solution was infused over period of 6 hours.

Immediately after the end of infusion, the experimental animal and the control were sacrificed simultaneously From each animal, foetus was delivered abdominally and transferred to paraffin bath f 37°C for recording of the ECG. Lead I was recorded with an Elema Mingograph. The remaining foetuses of each rabbit were transferred, with intact membranes, to physiological saline (37°C), in which they remained until the appearance of ECG changes implied that the time was suitable for taking myocardial specimens for fine structure studies.

For these studies, small blocks of papillary muscle from the left entricle were immersed in 2% collidine buffered (pH 7 4) osmium tetroxide (OsO_4) at 0–4°C for 2 hours. The tissues wer dehydrated in ethanol solutions of rising strength. They were then immersed in propylene oxide, and finally embedded in *Epon*. The sections were stained with lead citrate. They were inspected and photographed in Siemens Elmiskop I electron microscope at magnifications ranging from 3000 to 10,000 times. The negatives were photographically enlarged as desired.

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RESULTS

Initially the ECG appeared normal in both the control and the experimental foetuses.

The fine structural appearance of the myocardium was essentially in conformity with that in previous studies of adult mammalian tissues (9 13 14 16, 17) (Fig. 1). It was, however noted that the foetal mitochondria were more irregular in size and shape than in adult animals. Mitochondrial matrical granules were seldom observed.

Glycogen in monoparticulate form was abundant in the cytoplasmic ground substance. In some areas, glycogen particles were also visible in the myofibrils. No clear-cut difference was present between foetuses of glucose-treated and of untreated mothers with respect to the apparent amount and packing of glycogen in particulate form. Judging from previously published pictures (9 13 14 16, 17), the amount of particulate glycogen was much larger in the foetal than in normal adult cardiac muscle cells.



Fig. 1 Normal. Note abundance of glycogen particles in cytoplasmic ground substance. *m*, Mitochondria, *mf* myofilaments, *N* nuclei. 15,000.

After *38 minutes'* anoxia, pathological changes became conspicuous in the ECG of the control foetus (C in Table 1 and Fig. 2) but were barely recognizable in the ECG of the foetus of the glucose-infused rabbit (G in Table 1). The heart of the control foetus beat at a higher rate, but some of the contractions were blocked, whereas the heart of the experimental foetus was still beating perfectly regularly. In the control foetus, the QRS had low potential and the S-T junction was depressed, implying myocardial damage.

These changes in the ECG of the control foetuses corresponded to a diminished amount of particulate glycogen in the cytoplasmic ground substance (Fig.) Occasionally mitochondria showed irregular pale areas of the matrix, with some distortion of the adjacent cristae. In the experimental foetus, no such mitochondrial change could be observed. The amount of particulate glycogen in the cytoplasmic ground substance was, however reduced.

After *45 minutes* anoxia, the ECG of the control foetus (C in Table I and Fig. 3) had deteriorated further. The ultrastructural changes just described had become more marked. Furthermore, changes were present in the sarcoplasmic reticulum (dilatation and fragmentation), as well as in the nuclei (margination of chromatin). In

Table 1 *Heart rate and duration of ECG components after varying periods of anoxia*

Anoxia (min) ...	38		45		56		62	
Foetuses ...	C	G	C	G	C_2	G_2	C_3	G
Heart rate (beats/min) ...	60 irregular	50 regular	60 irregular	50 regular	58 (atrial) 29 (ventricular)	36	57 (atrial) 19 (ventricular)	36
QRS (msec)	28	36	28	36	156	40	32	60
PQ (msec)	124	104	124	104	420	208	400	240
P (mV)	0.13	0.25	0.13	0.25	0.06	0.06	0.10	0.10
R (mV)	0.19	0.56	0.19	0.56	0.20	1.0	0.10	0.30
S (mV)	0.30	0.90	0.40	0.90	1.0	0	0.90	0
T (mV)	0.10	0.40	Isoelectric	0.40	Isoelectric	0.20	Isoelectric	0.30 (neg.)
ST (mV)	0.10	Isoelectric	0.10	Isoelectric	0.70	Isoelectric	0.60	0.05



Fig. 2. 38 minutes. Control. Pronounced loss of glycogen particles. Note slight pallor of the matrix in some mitochondria (arrows). Myofilaments (*mf*) appear unaltered. *N* Nuclei. ×13,000

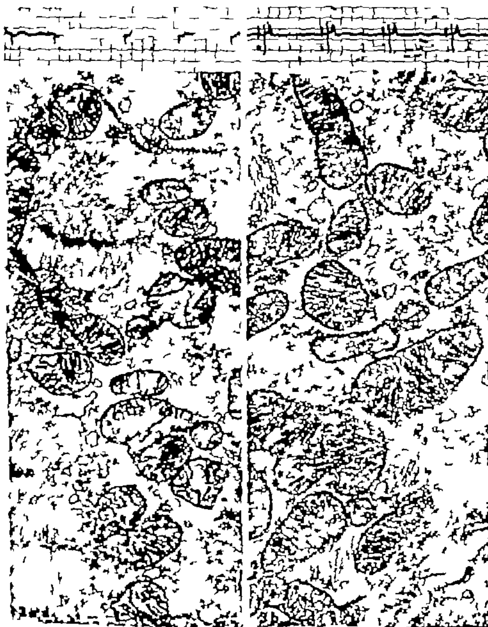


Fig. 3. 45 minutes. (*a*) Control (C_1). Many mitochondria show pallor of matrix, disorganization of cristae, and moderate enlargement ("swelling"). Glycogen particles are sparse ×16,000. (*b*) Experimental animal (G_1). Mitochondria appear essentially unaltered. Glycogen particles are few ×26,000.

the experimental foetus (G_1 in Table 1 and Fig. 3 *b*) on the other hand, there were still no changes in the ECG. Most of the glycogen particles had disappeared, whereas mitochondria and other cytoplasmic organelles appeared unaltered.

An atrio-ventricular block had developed in the control foetus after *56 minutes* anoxia (C_2 in Table 1 and Fig. 4 *a*). Both the atria and the ventricles contracted regularly, the atria at a rate of 58 beats/minute and the ventricles at 29 beats/minute. The heart rate of the experimental foetus had fallen to 36/minute, but otherwise the ECG still appeared normal (G_2 in Table 1 and Fig. 4 *b*). In the control foetus, the ultrastructural changes

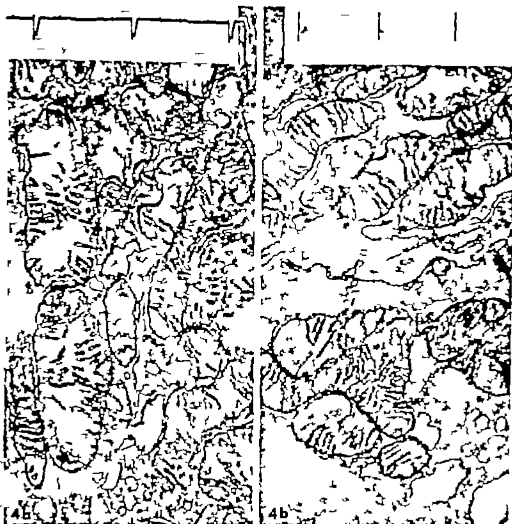


Fig 4 56 minutes (a) Control (C_1) Mitochondria are markedly swollen and show disorganization of cristae. x22,000. (b) Experimental animal (G_1). Some mitochondria (lower portion of the picture) are unaltered while others (upper portion of the picture) appear slightly swollen x22,000.

observed already after 45 minutes had become even more pronounced. At 56 minutes, pathological myocardial changes in mitochondria and sarcoplasmic reticulum were also observed in the foetus of the glucose-infused rabbit.

After *62 minutes* anoxia, the ECG of the control foetus had deteriorated still further (C_2 in Table 1 and Fig. 5 *a*). The myocardium of the experimental foetus was also damaged, as judged by depression of the S-T junction (G_2 in Table 1 and Fig. 5 *b*).

In the *myocardium of the control foetus* subjected to more than one hour of anoxia, there were two types of mitochondrial changes not previously observed (Fig. 5 *a*). They were (1) appearance of focal dilatations of cristae, and (2) occurrence of large irregular densities in the matrix. At this time, considerable distortion of other cytoplasmic organelles was also seen. Although present in most of the sections studied, glycogen particles were rare, and widely spaced in the pale cytoplasmic ground substance. Cytosomes appeared unaltered.

In the *myocardium of the experimental foetus*, the alterations in mitochondria and sarcoplasmic reticulum first observed after 56 minutes were pronounced after *62 minutes* (Fig. 5 *b*). At this time, only occasional glycogen particles were pre-



Fig. 5. 62 minutes. (*a*) Control (C_2). Pronounced disorganization of cytoplasm. Some mitochondria show presence of irregular matrix densities (*d*) and widening of intracristal spaces (arrows) ×18,000. (*b*) Experimental animal (G_2). Mitochondria are swollen but lack matrical densities. Intracristal spaces are of normal width. Note dilatation of sarcoplasmic reticulum (*sr*) ×18,000.

sent in the cytoplasmic matrix. Mitochondria were swollen with pale matrix and partly disorganized cristae, while the sarcoplasmic reticulum was dilated and fragmented.

After *72 minutes'* anoxia, the ECG of the control foetus showed no activity whereas some persisted in that of the experimental foetus, although its myocardium exhibited severe changes (Figs. 6 and 7). Thus, irregular widening and disorganization of mitochondrial cristae were visible, as well as the occurrence of irregular densities in the matrix. Swelling of mitochondria with pallor of the matrix was not prominent. Changes in other cytoplasmic organelles and the cytoplasmic matrix were similar to those seen at 62 minutes and later in the controls.

COMMENTS

The findings in this study suggest that fine structural alterations in heart-muscle cells during anoxia are delayed by preceding loading of the cytoplasm with glycogen. Occurrence of changes in cytoplasmic organelles, in particular in the mitochondria, together with pronounced loss of particulate glycogen, appeared to correlate with electrocardiographic changes. The observations sup-





Fig. 6. 72 minutes. Experimental animal. Mitochondria with matrical densities (arrows). ×24,000.

Fig 7 72 minutes. Experimental animal. Mitochondria showing irregular widening of intracristal spaces (arrows). ×32,000.

port the view that pretreatment of the mothers with glucose in order to build up large stores of glycogen in the foetal heart does, in fact, increase the ability of the heart to resist functional and morphological effects of anoxia. Such treatment appears to cause a delay in the onset of the morphological changes. The sequential development of the changes is nevertheless similar in the glucose-infused and control animals.

Early pathological alterations in the fine struc

ture of the muscle cells consisted of apparent swelling of mitochondria, with pallor of the matrix and disorganization of cristae. Functionally these changes were reflected by an atrio-ventricular block and depression of the S-T junction. Similar mitochondrial changes have been described in the early phases of anoxia and autolysis in several tissues (2, 8 9 20). They have also been reported to occur as a result of hypoxia and certain toxic influences on various cell types (for a review see e.g. (18). There is evidence from other tissues that these changes are reversible (2) The findings in the present study indicate that the alterations are compatible with retained—although somewhat impaired—contractile function of the cells. It is likely that this function is upheld by anaerobic glycolysis.

It is presently agreed that the dense granules in the cytoplasmic matrix, measuring approximately 200 A in diameter and stainable with lead-containing solutions, represent glycogen in particulate form (3, 4 15). Good correlation was usually present between the number of such granules seen in the sections and the amount of glycogen measured biochemically A decrease in glycogen during anoxia appears to be due to an increased need of anaerobic glycolysis. The parallelism between morphologically visible and biochemically measurable glycogen seems to support the view that glycogen in particulate form represents the main (and possibly sole) source of intracellular glycogen in the foetal heart. The decrease in glycogen was accompanied by a fall in heart rate (6).

Previous studies have suggested that the occurrence of amorphous matrical densities in mitochondria is an early sign of necrosis (5, 8, 9 18 20), and probably represents an irreversible change. In the anoxic foetal heart, the appearance of such densities coincided with widening of the intracristal space (dilatation of cristae) in many mitochondria. Although the latter alteration was not observed in necrosis of the liver *in vivo* (19 20), it has been observed in anoxic renal-tubule mitochondria (5), and may also be a sign of incipient necrosis. That these mitochondrial changes reflect severe and probably irreversible damage to the muscle cells is supported by the fairly pronounced alterations in other cytoplasmic organelles and in the nuclei.

The evidence derived from the ultrastructural observations indicated that the mitochondria, during the period of anoxia, underwent series of alterations in size, organization of cristae, and content of water in the matrix and the intra- and pericristal spaces. Similar alterations can be induced in isolated mitochondria *in vitro* by changing the osmotic and ionic composition of the suspending medium, and also by changes in metabolic steady state (1 7 10, 11 21). In the present study the earliest alteration was probably an increase in water content of the mitochondrial matrix. This appeared to be followed by dehydration of the matrix and "hydration of the intracristal spaces. These states apparently reflect changes in membrane permeability and/or ability for selective active ion transport over the different types of crista membranes and the mitochondrial envelope. Since matrix contraction and dilatation of intracristal spaces occurred later in the glycogen-loaded cells, it does not seem likely that the lowered pH was responsible for these alterations (12).

Both biochemically and structurally some glycogen was still present in the cells at the time of structural and functional breakdown (6) This appears to contradict the theory that lack of substrate for anaerobic glycolysis caused the severe changes at this time.

SUMMARY

The heart function, as recorded by ECG was correlated to the ultrastructural state of the myocardial cells.

The material consisted of 4 rabbit mothers Two were used as experimental animals, and were given 120 ml of 30% glucose solution intravenously on the 29th day of pregnancy The other two served as controls. After the infusion, the rabbits were sacrificed, and their foetuses removed by laparotomy One foetus was placed in a paraffin bath at 37 C for ECG recording, and the others were placed in physiological saline. The two parameters, i.e., ECG and ultrastructural state of the myocardial cells, were correlated at various times. Pathological changes in the ECG were found to be reflected in ultrastructural changes in the cells These changes were similar in the experimental animals and in the controls, but occurred earlier in the latter animals than in the former

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REVIEW ARTICLE

DE LANGE SYNDROME

A Study of Nine Examples

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Segregation of the mentally retarded into definable groups according to common features helps in delineating clinical entities for each of which a cause or at least a pathognomonic clinico-pathological marker would eventually be found by incessantly applying new methods of investigation. This was true, for example, in the case of Down's and Cri du Chat syndromes where the chromosomal abnormalities were discovered. In other recently described disorders bearing analogies to conditions with known chromosomal aberrations, no cause has yet been defined. One of these is the syndrome described by the Dutch paediatrician Cornelia de Lange in 1933 who called it Typus Degenerativus Amstelodamensis, after the city from which her first case originated (6). Not less than 213 cases[3] have since been mentioned in the literature. While most such reports were simply descriptive, a few recorded multiple endocrine hypofunction or inconsistent chromosome and biochemical abnormalities. To reexplore these and other aspects of the disease, a comprehensive study was made of 9 cases which is reported here.

SELECTION OF PATIENTS

Diagnosis of the syndrome rests mainly upon the recognition of combination of severe mental and physical retardation with characteristic cranio-facial discrepancies, specific patterning of hypertrichosis and congenital anomalies of the upper limbs and less frequently of other parts of the body. Some of the features are non-specific and some are shared with those of other relatively well established entities. Moreover formes frustes of the syndrome undoubtedly exist. Thus, hile it is usually easy after familiarity with the condition to spot classical case, diagnosis can sometimes prov difficult. Strict adherence to certain criteria had therefore to be observed in the selection of the patients for this study. Those lacking both of the following facial features (Fig. 1) were excluded; (*a*) eyebrows, either thick and bushy or thin arc shaped, meeting in the mid-line (synophrys), sometimes extending down the nose bridge in V-shaped manner and (*b*) overhanging curved long thin upper lip with absent philtrum, sometimes with mid-line small extension and corresponding notch in the lower lip.

CASE REPORTS

The birth weights ere all below 2720 g (6 lb). All the patients had feeding difficulties with recurrent vomiting throughout infancy into early childhood. They pursued growth course below the third percentile for weight and height, except case 9 (Fig. 2), and their teeth eruptions were markedly delayed. In Tables 1 and 2 some clinical data and common features found in tw or more patients are tabulated hilst Fig. 3 show their pedigrees. The following is description of other important aspects of individual cases.

Case 1

Sensations exactly like period" ere experienced by the mother at 4 eeks of pregnancy although unaccompanied by vaginal bleeding. Hyperemesis persisted through

The endocrine part of this study was presented at the 6th Annual Meeting of the European Paediatric Endocrine Society held in Israel in March 1967

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[3] A list of references and table of the chromosomal abnormalities reported in this syndrome can be provided on direct application to Dr J. M. Abraham, The General Hospital, Ashton-u-Lyne, Lancs., England



Fig. 1. Characteristic facies of 9 cases of de Lange syndrome.

Table 1 *Clinical data of the 9 cases of de Lange syndrome*

Parents' ages at time of birth of patient.

Case no.	1	2	3	4	5	6	7	8	9
Sex	M	M	M	F	M	M	M	F	M
Birth order	2	1	9	1	(2)	2	3	1	4
Birth weight (g)	1700	2466	2495	1728	2580	2350	2523	2154	2636
Gestation (weeks)	37	38	36	39	40	40	40	38	39
Father' age	33	25	33	21	27	22	20	31	31
Mother' age	33	21	31	19	21	22	18	28	31
Age of study (years)	2 6/12	8 2/12	7 4/12	2 1/12	5	7	4 6/12	9 9 12	10 12

Table 2. *Clinical features of 9 cases of de Lange syndrome*

+ = Marked, + = present; ± = mild, − = absent.

Case no.	1	2	3	4	5	6	7	8	9
Facies									
Synophris	−	+	+	+		−	−	−	−
Long upper lip	+		−	+	+	−	−		−
Anteverted nostrils	+	±	−	+ +	−		−	=	±
Depressed nose bridge	−	−	+	−		±	+	±	−
Hypertelorism	−	−	+	−	+		−		−
Anti-mongoloid slant	+	−	+	−	−	∓	−	−	−
Epicanthic folds	−	−		−	+	−	−	−	−
Corectopia[a]	−	−	−	−	+				+
Micrognathia	+	−		+	±	±	=	−	−
Prominent symphysis mentis	+	−	−	−	+	−			−
High arched palate	−	+	−	±			−		−
Skull									
Macrocephaly	+	+	+	−	−		−		
Brachycephaly	−	+	+	−	−	−			
Hands									
Proximal thumb		±	±						
Fifth clinodactyly								−	
Simian crease/s									
Tapering fingers		−	−			−			−
Finger clubbing									
Arms									
Limited elbow movements					−		−		−
Skin									
Hypertrichosis									+
Cutis marmorata				=					−
Rough dry skin									
Ginger-coloured scalp hair					−	−	−	−	
Feet									
Webbing (2 3) toes				−	±				−
Others									
Cryptorchidism and small penis				0				0	
Growling voice					−				
Heart murmur									
Initial hypertonus	−								

[a] Corectopia   eccentric pupils

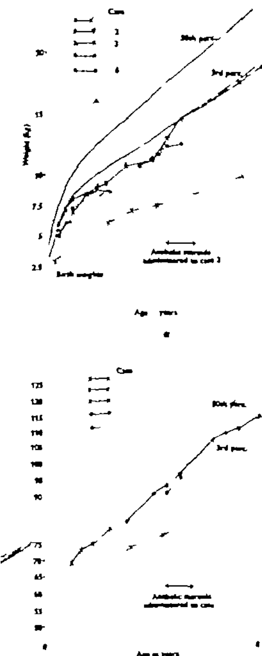


Fig Growth patterns: (*a*) weights and (*b*) heights of the 7 male patients plotted on growth charts for boys. The tw female patients followed the same trend on the appropriate charts.

the first 3 months. At delivery there was a comment about "placental insufficiency" by the attending midwife.

The patient was nursed in an incubator for weeks. At 3 days he had maximum indirect bilirubin of 17.6 mg/100 ml. At the age of 9 months generalised convulsions emerged which ere unresponsive to phenytoin sodium and later sulthiame (Ospolot) although partially controlled by Mysoline

His gums are now hyperplastic with asymmetrical dental eruption. Those on the right side are under developed and still embedded in the jaw while 8 teeth have erupted on the left side. He is severely myopic.

Case 2

This ginger-haired boy is the most forward of the group. H sat unsupported at one year walked at 2 years and said one or two words at 4 years. Intermittent courses of anabolic steroids (Anapolon) were given for one year from the age of 4 / years with slight increase in height and weight, but no disproportionate acceleration of skeletal age.

He has relative hypoplasia of the right side of the face. Both his middle fingers are deviated inwards.

Case 3

This patient, the most retarded in the group, was the 9th of 11 siblings born t gypsy first-cousin parents of low intelligence. A 19-year-old brother is severely retarded mentally short (3 below 3rd percentile) and microcephalic (head circumference 50.8 cm). H has small palpebral fissures, long lip with philtrum, micrognathia and bilateral simian creases. Otherwise there is no resemblance to the facies of de Lange syndrome. A sister who "looked definitely like the patient to a non-medical witness, was anophthalmic, spastic and mentally retarded.

He developed recurrent infections, with severe bouts of vomiting resulting in dehydration. At the age of 7 months diagnosis of agammaglobulinaemia was confirmed by the absence of visible band of gammaglobulin on protein electrophoresis, negative Coomb's anti-human globulin neutralising tests, absence of agglutinins to A and B cells (patient's blood group being O) and of adenoid tissue on X-ray of the naso pharynx.

At 2 / years he had repeatedly low blood standard bicarbonate levels. His hydrogen ion clearance index was 0.35 An intravenous pyelogram was normal. This suggested idiopathic renal acidosis and alkalies were prescribed. When repeated 8 months later the H⁺ clearance index was normal at 1.05 and the alkalies ere stopped.

H has bilateral corneal opacities which are certainly due to previous conjunctivitis. Both hands are cold and blue with marked finger clubbing (not found in the brother or mother). The grasp reflex is still present at nearly 7 / years, he follows objects, performs stereotyped movements ith his hands; but has no recognition of his surroundings H still shows hypo-gammaglobulinaemia on the protein electrophoretic strip (total serum proteins 6.35 g 100 ml: albumen 5 g, globulin 1.15 g). Coomb anti-human neutralisation test is positiv to dilution of 1 800 ith strong anti-A but weak anti-B agglutinins. Adenoid tissue is now present in the post nasal space on X-ray

Case 4

A right sided diaphragmatic hernia, containing the right lobe of the liver gall bladder duodenum, jejunal flexure and the right hepatic flexure, was repaired at the age of 2 eeks

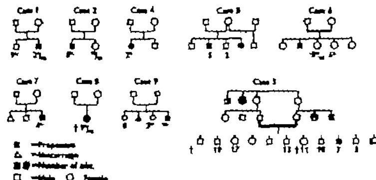


Fig. 3. Pedigrees of 9 cases of de Lange syndrome. Numbers beneath symbols indicate age in years. 0 = Stillborn or died soon after birth.

She has no webbing of the toes, but the 2nd toe overlaps the 3rd on the right side. The simian crease is also unilaterally present in the right palm.

Case 5

The mother, who is of low intelligence, had a normal 6-year-old son from a different negro father. Her first pregnancy from the patient's father (also coloured) terminated at 32 weeks due to placenta praevia. The baby lived a few minutes. He was noticed to have hypertelorism with epicanthic folds, beaked nose, low set ears, micrognathia, undescended testicles and horizontal palm creases. Autopsy revealed no abnormality.

Case 6

From the age of 5 months this patient was given thyroid tablets for nearly two years, with no improvement in his mental or physical status. From the age of 2 years and 9 months he had grand mal attacks which were treated initially with phenobarbitone then phenytoin sodium. In addition, at the age of 4 years he developed petit mal attacks which were controlled with corticosteroids. Both types of seizures terminated at the age of 4 years and 8 months.

His right leg is 1.5 cm shorter than the left, with an extensor plantar response on the latter side.

Case 7

Mother insists that she had been frequently kicked on the abdomen by the father throughout the first 6 months of pregnancy but there was no vaginal bleeding. The father, like his patient, has bushy eyebrows meeting over the root of the nose in a V-shaped manner.

In addition to the cardinal features, this patient has severe bilateral ptosis, hyperbrachycephaly, plagiocephaly, flat occiput and malformed ears. The soft tissues of the second and third toes are completely fused with transverse plantar creases bilaterally.

Case 8

A cleft soft and hard palate aggravated this patient's feeding difficulty. She continued to refuse solids and was severely constipated.

Both fifth fingers were conical in shape at their distal ends, with absence of dermal ridges and elongated cylindrical nails. In addition to the bilateral webbing between the 2nd and 3rd toes, there was dysalignment of the other toes.

The patient died shortly after an attack of severe diarrhoea and vomiting. Limited post-mortem examination showed no abnormalities in the heart, gastro-intestinal or genito-urinary tracts. The right and left lungs are quadri- and tri-lobate respectively with extensive patches of chronic lipoid pneumonia. Histology of the thyroid gland was normal.

Case 9

Because of a previous miscarriage, the mother was given unspecified "hormone injections" during the first three months of pregnancy. Similar injections had been administered in the preceding pregnancy which resulted in the birth of a normal girl. Her first pregnancy ended in a 3 weeks post-mature stillborn, post-mortem examination of which revealed no abnormality. Her first paternal cousin, 30-year-old male, is mentally retarded, has epilepsy and does not speak.

At 5 months the patient's serum protein electrophoretic strip showed decreased gamma globulin (albumin 4.8 g and globulin 1.8 g/100 ml).

INVESTIGATIONS

Routine

Except where indicated the following estimations were repeatedly found to be normal in all the patients.

Blood. Haemoglobin concentration, leucocyte count and differential, platelets, erythrocytic sedimentation rate, serum electrolytes, blood urea, standard bicarbonate, calcium, phosphorus, alkaline phosphatase, magnesium, G.O. transaminase and proteins (except for the hypo-gammaglobulinaemia in cases 3 and 9).

Urine. Specific gravity, mucopolysaccharide excretion, amino-acid paper chromatographic pattern and quantitative analysis (amino-acid nitrogen/total nitrogen ratio).

There was no excess of xanthurenic acid ex-

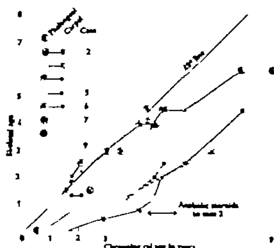


Fig 4 Skeletal development of 9 cases of de Lange syndrome. The encircled symbols indicate the skeletal age as judged by the development of the phalangeal and metacarpal bones. Note that the carpal bone development lags much further behind the chronological age.



Fig 5 X-ray of the right hand (case 4) showing short thick first metacarpal bone and small second phalanx of the 5th finger.

cretion following L Tryptophan loading (100 mg/kg body weight) Plasma amino-acid screening tests were normal as were plasma non-esterified fatty acids (NEFA) levels (except for case 6 in whom it was 1.54 mEq/l, or nearly double the maximal normal value) Wasserman reactions were negative. Cerebrospinal fluids (cases 1 3 7) were normal.

Radiological examination

The most consistent abnormalities involved the hands. There was discrepancy in the sequence of development of various centres of ossification, the phalangeal and metacarpal epiphyses being far more advanced than the carpal bones. Hence they were both plotted in Fig. 4 recognising the fallacy inherent in utilising solely the latter for the appraisal of bone age (8) Retardation of skeletal development was apparent in some cases, according to the standards of Greulich & Pyle (10)

In all cases the first metacarpal bones were proportionately short and thick and the 2nd phalanges of the fifth fingers were small, even rudimentary (Fig. 5). The right and left radial heads were malformed and congenitally dislocated in cases 6 and 8 respectively (Fig 6)

Skull radiograms showed normal pituitary fossae but enlarged dorsum sellae in nearly all the patients (Fig. 7) Apart from marked obliquity of the ribs in most patients (Fig. 8) skeletal surveys revealed minor abnormalities. Case 1 had an area of relative translucency and enlargement in the left ischial ramus. In case 7 there was a spina bifida of the seventh cervical vertebra and a synostosis between the left first and second ribs.

Central nervous system

Psychomotor development was severely impaired, speech maturation being especially defective. Other than case 2 who at the age of 8 years can link a few words, none of the patients can produce an intelligible sound. Though apathetic most of the time, a few recognised their parents and took limited interest in their surroundings. Their I.Q assessment was typically below 50 Cases 7 and 8 were more consistently hyperkinetic.

All were microcephalic their fronto-occipital circumferences falling 5–7 cm short of the 50th percentile for age (Table 3) Air encephalogram



Fig. 6 Radiograph of elbow joint (case 8) showing shallow sigmoid fossa and malformed (tapered) dislocated upper end of radius.

in case 3 showed dilatation of the lateral ventricles supporting a suggestion of underlying cerebral atrophy

Electro-encephalograms revealed definite diffuse abnormalities over both hemispheres in all the patients, with either fast or slow activities or both, and absence of alpha rhythm in some (Table 3). There were no focal or paroxysmal features, except in case 6 which had short generalised paroxysms of high voltage spike and wave activity

Chromosome (L. J Butler)

Using standard techniques (3) chromosome preparations were obtained from cultured blood leucocytes in all patients and from skin fibroblast culture in case 3 The karyotype patterns were normal in all cases.

Dermatoglyphs

A detailed study by Mr L. J Butler of the dermal ridge patterns (Table 4) revealed two main deviations from normal:

( ) *Finger tips*. There was a decreased number of whorls (3/90 = 3.3) and increased number of radial loops (21/90 = 23 %), the latter mainly in the middle three fingers, compared with a frequency of 26.1 % and 5 4 % respectively in normal controls (15).

(*b*) *Palms*. The most common finding was a



Fig 7 Skull X-ray (case at the age of 2 years and 9 months) showing an enlarged dorsum sellae. Note also the shallow posterior fossa



Fig 8 Chest X-ray case 8) showing marked obliquity of the ribs. Note also opacity of the posterior segment of the right lower lobe, which on post mortem proved to be lipoid pneumonia.

Table 3 *Head measurements, I Q.s and EEG findings in 9 cases of de Lange syndrome*

M–P = Merrill–Palmer S–B = Stanford–Binet, T M = Terman–Merrill G = Griffith, ? = no scale applicable.

Case no.	1	2	3	4	5	6	7	8	9
Head circumference (cm)	44.4	48.5	45	43.2	45.5	45	43.5	43.5	42.5
Mean normal for age (cm)[a]	49.5	52.2	51.8	49.2	50.8	51.6	50.7	53	46
Cephalic index	77.4	84.8	83.3	80	75	80	92	86	
I.Q	< 50[b]	55	< 20[b]	85[b]	47	30	17	< 20[b]	
Scale	?	T M	G	M–P	M–P	S–B	G	?	
EEGs									
Slow activity	+	–			+	+	+		
Fast activity		+	+	+			+	+	+
Absent alpha-rhythm		+	+		+			+	

[a] After Watson & Lowrey (40).
[b] Unreliable because of young age or difficult assessment.

loop from the *c* triradius into the third interdigital space (Fig. 9) It was present in $^5/_9$ left hands and $^6/_9$ right hands, compared with a frequency of about 45% and 50% in normal controls respectively (31). The *D* line exit was in the second interdigital space. Moreover the *b* triradius was absent on the right side in cases [illegible] and 8 and the *c* triradius in the left palm of case 8 No zygodactylous patterns were found, although in case 7 the triradii were displaced into the interdigital spaces. There was no clearly defined pattern in the thenar areas.

Endocrine studies

The following studies were carried out at approximately the ages indicated in Table 1

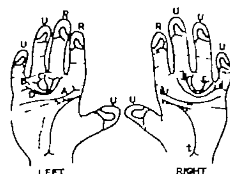


Fig 9 Reproduction of the main lines of the palms and finger tips (case 6) of typical dermatoglyph pattern. The triradii, *a–d* and and the main lines *A–D* are indicated on the right and left palms respectively. Note the simian crease on the left and the "interrupted" transverse line on the right, bilateral third interdigital loop from the *c* triradii and the radial loops on both index and the left middle fingers.

Thyroid function tests

Thyroid uptake of ^{131}I was measured at 2, 4 and 6 hours after oral administration (0.5 μC/kg body weight up to 8 μC) This was repeated 16 hours after an intramuscular injection of 5 units of thyrotrophic hormone (TSH) and again after 5 days stimulation (5 units daily) Serum cholesterol, protein bound iodine (PBI) and T_3 resin uptake were determined before and after stimulation with TSH. The results are shown in Table 5 and the 4-hourly uptakes are diagramatically represented in Fig. 10.

Some initial serum cholesterol levels were high, and came down substantially after TSH stimulation. Basal serum *PBI*'s and T_3 resin uptakes were normal, increasing significantly following stimulation in all the patients.

If the criteria for adults (14) are adopted, a *normal response would show an initial* 4 hour uptake of 11–31% of the administered dose, rising to more than 35% after the first dose of TSH with a further rise after more stimulation (as in case 5).

While the initial 4 hourly uptakes in all the patients were within the normal range the rise after the first dose of TSH was below this minimal figure in 6 patients. Of these, two (cases 3 and 9) showed a much greater rise than normal and another two cases (1 and 2) a further rise above the 35% level following further stimulation. This pattern suggests secondary hypothyroidism with its lack of endogenous TSH, partly a product of poor hypothalamic or pituitary TSH secretory responsiveness With the remaining two cases (4 and 7) there was no rise on further sti-

Table 4 *Dermal ridge patterns in 9 cases of de Lange syndrome*

W = whorl, U = ulnar loop, R = radial loop, A = arch, + = present, − = absent, ± = equivocal (interrupted line).

Case no.	Finger pattern Left V	IV	III	II	I	Right I	II	III	IV	V	Palm features: 3rd interdigital loop Left	Right	Simian crease Left	Right	atd angles (°) Left	Right	Sum
1	U	U	R	U	U	U	U	U	W	U	−	+	±	±	61	42	103
2	U	U	U	R	U	U	U	U	U	U	−	−	−	−	40	37	77
3*	U	W	U	U	U	U	R	U	W	U	+	+	−		79	95	174
4	U	U	U	R	U	U	R	U	R	U	+	+	−	−	42	44	86
5	U	U	U	R	U	U	A	R	U	U	+	+	−	−	47	44	91
6	U	U	R	R	U	U	R	U	U	U	+	+	−	±	31	47	78
7	U	U	R	R	U	U	R	R	U	U	−	−		±	36	40	76
8	−	R	R	R	U	U	U	R	R	−	−	+	−	−	96	105	201
9	U	U	U	R	A	U	U	U	U	U	+	−	−	−	100	88	188

* The dermatoglyphs of this case have been reported under case 8 of Smith (36).

mulation, a pattern of primary hypothyroidism. It should be noted however that, in spite of this, there was a nearly threefold increase in PBI following TSH stimulation. Case 4 had her basal

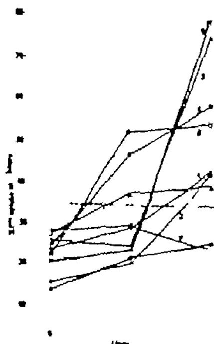


Fig. 10. Diagrammatic representation of the 4 hour thyroid percentage uptake of ¹³¹I before and after TSH administration. Numbers in the drawing refer to cases. Shaded area: normal basal uptake; interrupted line: 35% uptake (see text).

¹³¹I uptake 10 days after an intravenous ACTH test. The latter is reported to interfere with the thyroid uptake (35), but it is doubtful whether it is the sole explanation in this instance in view of the length of the intervening period. Likewise, gastrointestinal abnormalities are known to occur in this syndrome and interference with the absorption of the oral dose could conceivably account for the low thyroid uptake though there was no clinical evidence of malabsorption.

Hypothalamic pituitary-adrenal axis

(*a*) *Insulin tolerance tests.* 0.03 units of soluble insulin per kg body weight were injected intravenously and blood glucose was estimated by the glucose oxidase method in an auto analyser at 20 minute intervals. This small dose suffices to detect hypopituitary and or hypo-adrenocortical sensitivity and hypoglycemic unresponsiveness if present and avoids the hazard of expected hypoglycemic reactions with a higher dose in such cases. The results (Table 6) show that there was a normal response in 5 patients whilst the response was subnormal in one (case 6).

(*b*) *Urine steroid.* ACTH (25 units in 250 ml normal saline over 8 hours) and metyrapone ditartarate (30 mg/kg body weight in 250 ml normal saline over 4 hours) were administered intravenously starting at 10 a.m. Urine was collected over 24 hours on the day before the test and during the day of the test for estimation of 17 ketosteroids (17-KS) and 17 ketogenic steroids

Table 5 *Thyroid function studies in 9 cases of de Lange syndrome*

Normal values: PBI = 3 4–7.6 µg/100 ml ,, T3 resin uptake = 25–35 %

Case no.	1			2			3			4*		
TSH units	0	1 5	5 5	0	1 5	5 5	0	1 5	5 5	0	1 5	5
Serum cholesterol (mg/100 ml)	218		183	208		166	257			299		[illegible]
PBI µg/100 ml	7.2		12	3.4			3.6		12.8	4.6		[illegible]
^{131}I uptake												
2 hr	19	25	34	10	20	34	24	24	65	9	23	[illegible]
4 hr	24	30	43	16	21	43	26	25	75	14	22	[illegible]
6 hr	28	33	52	14	29	38	18	23	72	17	20	[illegible]
% T3 resin uptake	—	—	—	27		45	27		48.5	24.3		[illegible]

A different counter was used for cases 1 4 5 and 9

(17 KGS) There was an interval of not less than a week between the two tests.

Normally with the above methods applied to adults, a threefold to fivefold increase in urinary 17 KGS is expected following ACTH stimulation (41) and double the basal value following metyrapone (9) According to these criteria the responses to both ACTH and metyrapone (Table 7) were normal, although the basal values of 17 KGS were very low averaging between a quarter and half the normal mean values for age in our laboratory Although case 3 had the lowest basal value for age, when corrected for size, it fell within the usual range of the group.

The 17 ketosteroid urinary excretion was also low but less markedly so than the 17 ketogenic steroid excretion.

(c) *Plasma cortisol levels.* Venous blood was collected in the morning after a 10 hour fast and at 30 minute intervals following the intramuscular injection of 10 units of soluble corticotrophin (ACTH) or 10 units of lysine-8-vasopres-

Table 6. *Response to hypoglycaemia induced by intravenous insulin injection in 6 cases of de Lange syndrome*

Bl. Glu. = Blood glucose levels in mg/100 ml H.G H = plasma Human Growth Hormone in nanogram/ml.

Time (min) Case		0	20	40	60	90	120
1	Bl. Glu.	55	43		52	51	49
	H G H.	1	14		8	5	
3	Bl. Glu.	58	37	64	63	55	60
6	Bl. Glu.	72	53	48	62	66	63
7	Bl. Glu.	69	37	73	76	83	81
8	Bl. Glu.	67	57	60	63	65	70
	H G.H.		17	21		17	
9	Bl. Glu.	51	34	39	41	51	51

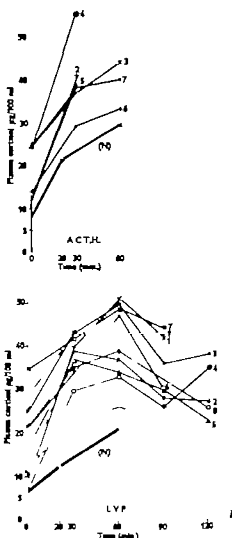


Fig 11 Plasma cortisol levels following the injection of 10 units of (*a*) ACTH, and (*b*) LVP compared with the mean normal values for children (*N*) of Wal *et al.* (39). Numbers on the curves refer to cases. Interrupted line = normal adult response to 10 units of LVP intramuscularly (17).

			6			7			8			9*		
0	1 5	5 5	0	1 5	5 5	0	1 5	5 5	0	1 5	5 5	0	1 5	5 5
4		103	200		125	159		188	155			171		94
5.1		13 6	6.1		9 0	3.9		10.0	4.4		14 8	5 1		14.2
9	37	45	24	38	25	28	28	23	22	46	54	14	16	76
5	47	59	28	38	40	28	30	25	23	53	55	21	24	79
8	53	68	29	—	40	24	29 5	36	20	54	64	24	31	81
67		41 4	32		43.5	22.5		34.5	26		32.5	29 1		34.5

sin (LVP). Plasma cortisol was assayed by the fluorimetric method of Mattingly (25). An interval longer than a week intervened between the two tests. LVP injection was followed by the usual skin blanching, but no other untoward effects.

Fasting plasma cortisol levels were high (mean 19 0 μg/100 ml, range 7–35 μg/100 ml) with an absolute average rise of 21 μg and 24 μg/100 ml within the first hour following ACTH and LVP injections respectively (Fig. 11) Judging by the mean values obtained from 48 normal children reported by Wal and associates (39) the rise in plasma corticol levels following ACTH was on the upper limits of normal, and all the patients were hyper-responsive to LVP It should be pointed out, however that these workers gave smaller doses: 5 units of ACTH and 0.5 units per year of LVP by intravenous infusion over one hour

(*d*) *Growth Hormone* Human Growth Hormone was assayed following glucose loading (16) by the double precipitation method as modified by Hartog and co-workers (1_). The plasma levels during the reactive hypoglycaemic phase after oral glucose administration (1 75 g/kg) are listed in Table 8 with the corresponding blood glucose values. They fall within the normal range of 10–30 nanograms/ml (5)

DISCUSSION

Diagnosis

No pathognomonic findings have emerged from this study The diagnosis remains a clinical one, depending mainly upon the summation ("Gestalt) of several more or less constant features headed by the two stigmata used in our arbitrary routine of selection. Two additional signs, however may be helpful.

(*a*) *Radiological* The short thick first metacarpal bone found in all the present cases and already referred to by previous workers (38, 22). Kurlander & DeMyer (21) regarded a combination of a short first metacarpal, hypoplastic middle phalanx of the fifth finger and a deformity of the proximal radial metaphysis with subluxation as probably specific for the syndrome

(*b*) *Dermatoglyphic* The finding of radial loop/s on the 3rd and 4th finger tips and the third interdigital loop In an analysis of the dermatoglyphs of 21 patients with "variation in

Table 7 *24 hour urine steroid excretion before and after ACTH and Metyrapone administration in 5 cases of de Lange syndrome*

Case no.	1		2				4		5		6
Test	0	ACTH	0	ACTH	0	ACTH	0	ACTH	0	ACTH	0
17 KGS (mg)	0.4	4.85	1 54	7 35	0 65	3 1			0 8	2.4	
17 KS (mg)	0 25	1 75	0 64	0.93	0 2	0 6			0 6	1.2	
Test	0	Met	0	Met	0	Met	0	Met	0	Met	0
17 KGS (mg)	0.95	2.1	0 88	_25	0 4	0 8	1 1	2.3	0 5	1 1	1.6
17 KS (mg)	0 4	0.7	0 21	0.31	0 3	0 4	0 1	0.2	0.3	0 5	0.4

Table 8 *Glucose tolerance tests and plasma human growth hormone estimations in 9 cases of de Lange syndrome*

Bl. Glu. = Blood glucose in mg/100 ml. H.G.H. = plasma Human Growth Hormone in nanogram/ml. H.G.H in cases 1 and 8 was estimated during insulin-induced hypoglycaemia (see Table 6).

Time (hrs). Case		0	1/2	1	1 1/2	2	3	4	5
1	Bl. Glu.	71	64	117	78	83	42	40	50
2	Bl. Glu.	53	65	87	90	88	68	51	60
	H.G.H						< 4	16	9
3	Bl. Glu.	50	102	122		106	54	58	46
	H.G.H.						20	6	<4
4	Bl. Glu.	54	108	95	108	86	56	51	52
	H.G.H.						25	7	12
5	Bl. Glu.	52	89	82	70	68	57	42	43
	H.G.H.						3	24	4
6	Bl. Glu.	40	192	204	180	198	147	71	
	H.G.H.						12	8	
7	Bl. Glu.	76	140	105	97	109	83	82	88
	H.G.H.						20	<4	<4
8	Bl. Glu.	*60*	*266*	*180*	*156*	*78*	*48*	*42*	*54*
9	Bl. Glu.	48	105	76	96	57	42	46	49
	H.G.H	3		14		6	8	8	<5

the certitude of the diagnosis Smith (35) also concludes that the dermal ridge pattern is a useful criterion.

In this context there were two interesting cases among the patients excluded from this study for lack of one or the other of the two facial facets (namely the synophris and the characteristic lip). One patient's lip was atypical but the major proximal part of his first metacarpal bone was missing with the thumb attached to the hand by soft tissues only The other with only a mild synophris, had bilateral complete soft tissue syndactyly of the 3rd and 4th fingers, a condition previously described in this syndrome (24 4) This may reflect a gradation in the severity of this congenital defect from the mild dermatoglyphic abnormality to the complete bony fusion of the two fingers (found in one of the fatal neonatal group of one of the authors)

Other aspects of the syndrome are also demonstrated in this series. the asymmetry of the defects on the two sides of the body (cases 1 4 and 6) and the congenital abnormalities of the gastro-intestinal tract (cases 4 and 8)

The low urine specific gravity hypo-amino-aciduria and elevated plasma glutamic acid level reported by Ptacek *et al.* (32) have not been confirmed, but different quantitative urine analysis standards were adopted and only plasma screening for amino-acids was possible The explanation does not seem to lie in the difference in the ages of the two groups. Although most of the patients of Ptacek *et al* were under .. years of age, one was 5 1/2 years old and moreover the same biochemical finding was reported in a 7-year-old patient with mild mental retardation (26).

Hypogammaglobulinaemia was found in case 3 and to a lesser degree in case 9· but there was slowly progressive improvement with age.

Etiology

The consanguinity of the parents in case 3 is of doubtful significance in view of their special ethnic group On five previous occasions this was mentioned but in two cases it existed in the maternal and paternal *grandparents* of the patient (42, 29 respectively) and in another it involved the parents of a presumptive case (according to the description of the relatives) whose paternal grandmother was the sister of the acutal patient's paternal greatgrandmother (?) More recently Pearce *et al.* (30) reported two unrelated instances with Greek and Maltese first cousin parents. The presence of synophris, a common occurrence among normal people of southern European origin as rightly pointed out by the authors, might have biased them in including these two cases, who otherwise bear remote resemblance facially to the de Lange syndrome. This conspicuous rarity of consanguity militates against an autosomal

recessive mode of inheritance, a hypothesis maintained by Opitz and associates (28).

As far as possible both parents and sibs of our patients were examined. Abnormalities were found in cases 3, 5 and 7 as described above. The high frequency of minor anomalies or central nervous system defects in other family members (see 28) lets us at least two interpretations: dissociated inheritance of individual anomalies stemming from incompletely recessive gene in a heterozygote carrier or a dominant gene with variable penetrance.

No environmental cause has yet been incriminated. That the syndrome is most probably mediated through a gene defect is supported by the report of the condition in three pairs of monozygotic twins (28, 4, 20) though one pair (20) might well be examples of Rubinstein-Taybi syndrome (33) according to the published photographs.

Out of about 1.6 cases known to have been subjected to chromosomal analysis, 19 showed some abnormality. It is by no means certain that all these cases are in fact genuine examples of the syndrome for lack of adequate photographic reproduction. With this reservation, however, the incidence of such abberrations, though inconsistent, would appear significantly high. Nevertheless, the present series confirms the generally accepted view at present that chromosomes are normal in this syndrome although small abnormalities cannot be regarded as entirely excluded by the techniques presently available.

Endocrine studies

Thyroid studies did not reveal a uniform malfunction. There was no clinical correlation even in the cases which showed the lowest radio-active iodine uptake. This is also borne out by the ineffectiveness of therapeutic trials of thyroid. Direct estimations of TSH were not done, but it would be inferred from the results of the tests in cases 3 and 9, and probably in cases 1 and ..., that there was a selective or isolated deficiency of TSH secretion by the pituitary, possibly the result of some interference with its hypothalamic thyrotrophin-releasing integration within the centre believed to be located in the vicinity of the median eminence.

The very low urine steroids permit no conclusion as to the function of the hypothalamic-pituitary-adrenal axis based upon the response to ACTH and metyrapone administration. The latter in any case, is an unreliable test in this regard. The non-correlation between the low urine 17 KGS's and the high resting plasma cortisol levels is difficult to explain, unless it stems from the stress inflicted in obtaining the venous samples. Were this so, it would in itself indicate a normal response by the hypothalamic-pituitary-adrenal axis. Alternatively it can be due to impaired hepatic clearance of cortisol (although no other impairment of hepatic function was apparent and the serum transaminases were normal) or increased plasma protein binding. On the other hand, high resting values of blood corticoids are observed experimentally when the hypophysis is isolated from cerebral connection (7).

The similarly high responses to both ACTH and LVP would suggest that the latter provoked a maximal response because of the large dose administered. On the other hand it may indicate a lesion in the hypothalamic area. The site of action of LVP is still uncertain. Hedge *et al.* (13) exploring this by intravenous, intrahypothalamic and intrapituitary injections in the rat, concluded that LVP appears to cause ACTH release indirectly by stimulating the secretion of a corticotrophin releasing factor from the median eminence region of the tuber cinereum. It may well be that this part of the hypothalamus was hyperresponsive in our patients because it is released from an inhibiting effect of a higher centre.

It is interesting to dwell for a moment upon the possible role of hypothalamic lesions in hypertrichosis. Kennedy (18) mentioned a 28 year old woman who developed severe hypertrichosis of the face and arms following encephalitis. List (23) recorded the case of a girl, who at 19 years of age developed hypertrophic gums, "hirsutism and striae over the body" among other signs and symptoms of hypothalamic upset and was found on post mortem six years later to have a pituicytoma infiltrating the tuber cinereum, upper part of the infundibulum and floor of the third ventricle. Besides these two cases of acquired hypertrichosis, Snyder (37) described two young girls with gingival hyperplasia, hirsutism and convulsions. One of them was mentally normal, but had eyebrows thick and bushy meeting above the nose. Because of the similarity of these

features to those of diphenylhydantoin intoxication, which is thought to be mediated through a diencephalic disturbance, the writer attributed the condition in his two patients to a similar localisation of pathology Direct evidence for this was lacking other than the symmetrically slow pattern EEGs. But this abnormality also found in most of our patients as well as the fast activities and the absent alpha-rhythm, are non-specific. On the other hand case 6 showed generalised paroxysms of high voltage spike and wave pattern, a record compatible with a lesion in the vicinity of the hypothalamic region. It should be recalled that this patient also had high plasma NEFA level and abnormal glucose and insulin tolerance tests, findings which can express hypothalamic dysfunction (19) Hypertrichosis (this term is advisedly used rather than hirsutism as the latter usually indicate specific endocrine hair distribution) is also one of the manifestations of generalised lipodystrophy a syndrome complex characterised, in addition to the complete absence of adipose tissue, by hyperlipemia, hyperglycemia without ketosis, hepatosplenomegaly and gigantism, and attributed by some to a lesion in the diencephalon. Likewise the persistance of cutis marmorata may possibly indicate an immaturity of a central vasomotor centre

The above argument justifies the speculation a lesion in the hypothalamic area. There is no specific mention of the examination of this area, macroscopically or microscopically in the few available brain necropsy reports from the literature, although a cyst of Rathke's pouch was found on post mortem in one case (1) and endocranial calcification in the suprasellar region was reported in another (27) The lateral ventricular dilatation linked to microcephally supports some form of underlying cerebral atrophy as a result of a primary embryopathic defect of cerebral maturation either gross or microscopic as in the paucity of ganglion cells reported by Hart *et al* (11). On the other hand it could be the product of a localised disturbance of neuronal metabolism as evidenced by the disorder of myelinisation (32) and gliosis (34) or the inhibition of cerebral growth by a more generalised metabolic disorder In either way the function of one or more hypothalamine integration is likely to be encroached upon.

SUMMARY

A comprehensive study of 9 typical cases of de Lange syndrome revealed no pathognomonic marker but two helpful aids to the essentially clinical diagnosis: short thick first metacarpal bone and suggestive dermatoglyphs. The series includes an example with parental consanguinity but the mode of inheritance remains uncertain. Endocrine investigations showed normal to high hypothalamic pituitary-adrenal axis response, with six of the nine examples showing evidence of hypothyroidism on functional testing, secondary in four and primary in two. Abnormality within the hypothalamic area or affecting its connections with higher centres, perhaps as part of a diffuse brain pathology could explain some of the findings in this syndrome

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CASE REPORT

CONGENITAL HAEMANGIOENDOTHELIOSARCOMA OF THE LIVER

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A fatal case of primary haemangioendotheliosarcoma of the liver in a 1-year-old boy has previously (in 1950) been reported from this hospital by Steinicke (12). This is a rare tumour and its presence at birth, with confirmed malignancy does not appear to have been described before (7 9 13).

CASE REPORT

Gentofte Hospital, Paed. Dept. Twin B, born 13.12.1966.

A boy twin B, was transferred immediately after birth private nursing home One previous pregnancy uncomplicated and the child in good health. Before her pregnancy the mother was given oxyprogesterone for 3 menstrual cycles. The pregnancy had been uneventful apart from minor vaginal bleeding in the 2nd month. During labour she received 2 units oxytocin and 25 mg camylophine chloride, and the membranes were ruptured when the orifice measured 5 cm.

The twin delivery was uncomplicated. Twin A was in the vertex presentation, and twin B, born 10 min later presented by the breech. The twins were dizygotic.

Twin A. Birth weight 1950 g, length 43 cm. At admission, aged about 1 / hours, he had distressed and grunting respirations, circumoral cyanosis, and retraction of the costal margins and lower sternum. He did fairly well in an incubator during the first 24 hours, but then deteriorated and died 2 days after birth.

Post-mortem diagnoses. Prematurity mild cerebral oedema, lungs brownish red, in places atelectatic, and did not float on water No other abnormalities In particular normal liver.

Twin B Birth weight 2500 g. More debilitated than twin A at admission. Greatly grunting respiration, generalized greyish-pale hue, and considerable retractions of the intercostal spaces, costal margins, and lower sternum as well as dilated nostrils. A swelling in the right abdomen was noticed immediately A general X-ray view of the abdomen showed the intestine to be shifted to the left, with sparse intestinal gas.

As the diagnosis was in doubt—Wilms tumour neuroblastoma, hydronephrosis, and teratoma were considered—the right hypochondrium was punctured and yielded cloudy fluid with small fragments of tissue.

Preliminary microscopic examination revealed an embryonic, malignant tumour. *Owing to the infant's condition* it was then decided to abstain from surgery

The patient was placed in an incubator and given oxygen. Glucose 5% was administered i.v with an addition of sodium bicarbonate because of severe acidosis. At the age of few hours the patient had respiratory arrest, but was soon restored by artificial respiration 9 hours after birth he had cardiac arrest, but was given external cardiac massage and was thereafter intubated and provided with I.P.P.B in an Engström respirator Microscopy of the tumour was not available at that time There was transient improvement, but then again slow deterioration, and death occurred about 15 hours after birth.

Other findings: Hb. 16.3 g/100 ml, R. B C. 4.5 mill/mm haematocrit 60%, blood urea 28 mg/100 ml, acid *base-status. varying degrees of acidosis. Blood group* A, Rh pos direct Coombs test negative. Chest radiography A shadow in the left upper lobe and smaller shadow apically in the right upper lobe. X-rays of abdomen *vide supra.*

Post-mortem diagnoses: Malignant tumour 10×9×9 cm, in the right lobe of the liver Hepatic capsule intact. The tumour was vascular and brittle. In the left adrenal gland there was haemorrhage, 1 1 cm. The lungs were grossly normal, reddish brown, aerated, and with no focal changes. Microscopic examination showed hyperaemic, partially atelectatic pulmonary tissue Normally developed foetal brain without abnormal changes.

Microscopic examination of the hepatic tumour: Primitive haemangioendotheliosarcoma of the liver. The tumour was made up of roundish or oval cells measuring between 10 and 20–25 µ. Fairly little cytoplasm. Nuclei relatively large, hyperchromatic. Nucleoli distinct. The

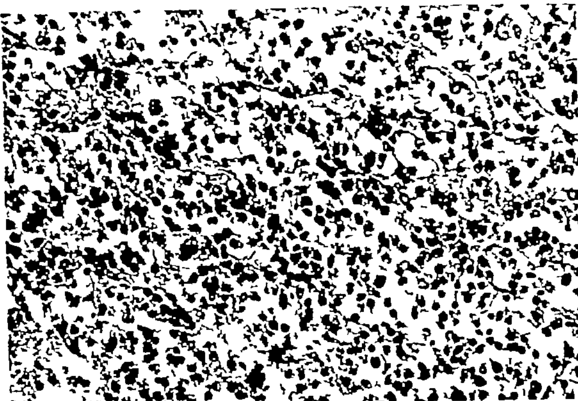


Fig. 1. Hemangioendotheliosarcoma hepatis cong. (1 400).

chromatin was distributed in very small granules and delicate threads. There were prophases as well as mitoses. Chromosomes slender. The cells formed thin or somewhat thicker cords, surrounding spaces filled with red cells. Around the tumour there was hepatic tissue in which the sinusoids were surrounded by the named cells. The liver cells, with delicately granular cytoplasm, were compressed by these tumour cells. In several sites haemorrhage, splitting the tissue (Sæborg Ohlsen).

DISCUSSION

During the past few decades the relative share of neoplasms in childhood mortality has considerably increased (2). However congenital malignant tumours are rare (9, 10, 13). According to Kauffman & Stout (9) a congenital tumour is taken to be a growth observed at birth or in the course of the first week of life. However most tumours in children occur before the age of years. It is believed that in most cases they consist of embryonic remnants and are thus in actual fact congenital (2).

In 1940 Wells (13) published an analysis of all reported cases of congenital malignant tumours. He found only 66 to be definitely malignant. The great majority were sarcomas. There were hepatic sarcomas definitely malignant and congenital according to the above definition. Furthermore, he found a congenital malignant haemangioma which had been reported by Veeder & Austin in 1912.

Another 5–10, presumably congenital, malignant hepatic haemangioendotheliomas did not manifest themselves until the age of 3 months. Kauffman & Stout (9), in 1964 reviewed the literature on congenital mesenchymal tumours, adding a few cases of their own. From this material they excluded all haemangiomas, benign haemangioendotheliomas, and lymphomas owing to their common occurrence. Kauffman and Stout found total of 120 congenital tumours, 2 of which were malignant haemangioendotheliomas—affecting the connective tissue, one in the arm and the other in the leg. They did not describe confirmed, malignant congenital primary tumours of the liver. Dorothy H. Andersen (?) analysed the share of tumours in the admissions to the Babies Hospital, New York, during the period 1935–1951. Among approximately 80 000 admitted children

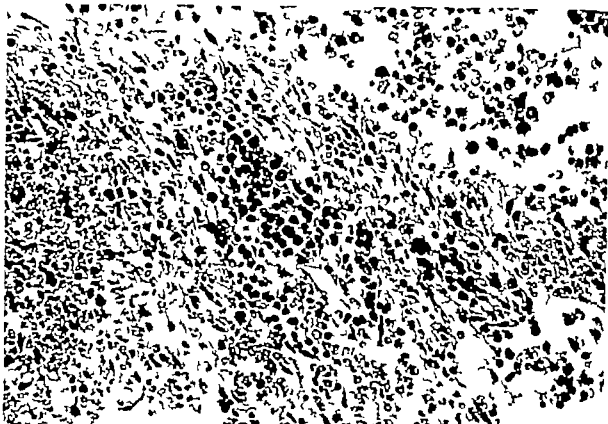


Fig Hemangioendotheliosarcoma hepatis cong. (1 400)

75 had malignant tumours (about _% of all ad ¹ children). Of these tumours 3 proved to be ' nary carcinomas (hepatomas) one a carcinoma of the bile ducts, and one an angiosarcoma in a _-month-old boy

There have been several case reports and reviews on haemangioendotheliomas of the liver (1 3 4 5 6, 8 10, 14 15) However the majority have been in adults, and the few malignant ones in children have been from an age older than our case. Finally Ishak & Glunz (7) in Cancer for 1967 reviewed 47 cases of primary epithelial tumours of the liver in children seen through the past 40 years in the "Hepatic Branch Armed Forces Institute of Pathology and the Veterans Administration Central Laboratory for Anatomic Pathology and Research ATIP" Washington D C. Only 2 of these cases had been published previously There were 35 hepatoblastomas and 12 hepatocarcinomas. Most of the hepatoblastomas had manifested themselves during the first year of life, the majority of the carcinomas after the 5th year There was a considerable preponderance of boys. None of the hepatoblastomas was of the structure seen in the present case. Diagnosing a hepatic tumour during childhood is usually difficult. The most common congenital abdominal swellings are due to Wilms tumour neuroblastomas, congenital hydronephrosis, and various teratomas (5) A thorough physical examination may be supplemented by diagnostic aids such as X rays with contrast medium in the colon X-rays of the stomach and small intestine, intravenous pyelography various tests of liver function, and possibly puncture and biopsy If it is of vital interest to obtain an immediate diagnosis or diagnostic hint (5) Making the diagnosis of primary hepatic tumour in a child as early as possible has acquired increasing interest in recent years, as the results of liver resections have favourably altered the prognostic outlook (5 6, 7 8)

Surgical treatment has proved superior to radiotherapy and it must be recommended to do exploratory laparotomy and obtain biopsy as early as possible, if there is a suspicion of hepatic tumour (7)

In our patient, who had severe respiratory failure leading to respiratory as well as cardiac arrest, the puncture was done in order to assess the advisability of and indication for abdominal surgery should the general condition later render this possible.

SUMMARY

A case of haemangioendotheliosarcoma of the liver in a newborn boy is reported. The patient died 15 hours after birth. The autopsy findings are described, and the rare occurrence of this tumour is discussed. It does not appear to have been previously diagnosed immediately after birth. A few cases have manifested themselves in the 2nd–5th month of life. Recent improvement in the prognosis for patients with hepatic tumours treated by surgery is mentioned.

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PROCEEDINGS OF PEDIATRIC SOCIETIES

FINNISH PEDIATRIC SOCIETY

Meeting Feb. 18 1967

Bertil Lindquist (Lund): *Glucose-galactose malabsorption*

Monosaccharides with a specific stereostructure like glucose and galactose are rapidly absorbed through specific energy-consuming process, presumably via a carrier mechanism related to the sodium pump. Other monosaccharides cannot accumulate in the mucosal cells against a concentration gradient.

Monosaccharide malabsorption exists as congenital and aquired forms. (1) the only congenital form so far known is glucose galactose malabsorption, (2) the aquired forms are secondary to unspecific lesions of the intestinal mucosa in several intestinal disorders, usually combined with more less marked atrophy of the intestinal mucosa.

Glucose-galactose malabsorption is a hereditary disorder inherited in an autosomal recessive way. The clinical picture of this disorder is similar to that of lactase deficiency. Diarrhoea disappears, however, not until the infant is given a carbohydrate free diet or a diet containing only fructose as carbohydrate. Later on in life the diarrhoea has a tendency to subside and small amounts of milk and food items with a low starch content are tolerated.

The diagnostic procedure in glucose-galactose malabsorption is based on (*a*) fecal sugar measurements, (*b*) oral sugar loading tests, (*c*) intestinal intubation studies and (*d*) studies on intestinal biopsy specimens. The result of the intubation study after a testmeal containing glucose and fructose in mixture is specific for glucose-galactose malabsorption. It is furthermore apparent that *the absorption mechanism for fructose is separated* from that for glucose (and galactose). Possibly is fructose absorbed by the way of facilitated diffusion (i.e. via a specific carrier) which would explain the fairly efficient absorption that is seen under certain circumstances.

In vitro studies of intestinal biopsy specimens indicate that the specific glucose-galactose carrier is not functioning normally in glucose galactose malabsorption.

Meeting May 24 1967

Josef Houštěk (Prague): *Chronic non tuberculous diseases of the lungs*

Ole Wasz-Höckert & Alf Backman: *Present status of childhood tuberculosis in Finland*

In Finland, tuberculosis has become a disease of adults and old people during the last years. Considering the social structure of the modern society it is obvious that this shift towards older age groups only enhances the risk to which children are exposed. The annual number of fresh cases of tuberculosis in the whole population has remained almost constant during the last three years, although the total number of cases has decreased. The incidence of fresh cases of tuberculosis among adolescents and young adults is rather high in Finland. Therefore, at present there is no reason for changing the Finnish policy in respect to BCG-vaccination. On the other hand, intensified attention ought to be paid to the follow-up, to be carried out by repeated tuberculin tests, of those who have been vaccinated and to the need of revaccination. Adequately controlled chemoprophylaxis is a very efficient complementary measure, by which children living in a tuberculous environment can be protected. Great efforts are made

in all parts of Finland to get the situation under control in respect to tuberculosis of adults. When these efforts have led to a markedly decreased morbidity and the risk of infection has thus been reduced, the time has come for discussing a change of our BCG policy. The current situation and the prospects for the near future however do not justify any alteration.

Meeting Sept. 15 1967

Walter H. Hitzig (Zürich) *Congenital immunologic deficiency diseases with special ref ence to the Swiss type of agammaglobulinemia*

The normal defense mechanisms of the organism against antigens are of basic importance. The careful observation and analysis of congenital deficiencies has been of major importance for the elucidation of the normal defense mechanisms.

The observed anomalies involve either cells or humoral antibodies or both. Methodically and didactically it seems advisable to try to separate these two mechanisms, although their close interaction in the in vivo situation is obvious.

The first milestone in this development was the description of an isolated humoral immunodeficiency by Bruton (1952). Under the title of "Congenital agammaglobulinemia" he described two boys with recurrent infections, inability to form antibodies, complete lack of the γ-globulin fraction in electrophoresis and striking improvement therapy with γ-globulin his assumption that this was a congenital and sex-linked disease, has been confirmed by several hundred later observations.

It soon became apparent that the absence of the γ-globulin fraction was not a necessary feature of the disease. The term "antibody deficiency syndrome" coined by Barandun *et al.* describes in a more generalized way the basic defect of these patients.

In 1957 Swiss workers described 4 cases of "agammaglobulinemia" of a very malignant type. In spite of appropriate therapy all the patients died during the first year of life. They all presented severe lymphocytopenia, associated with severe anomalies of the lymphatic system and especially f the thymus. The combined deficiency of both the humoral and cellular defense mechanisms was assumed in the following cases. This could be confirmed in many other carefully investigated cases. A review of 71 cases investigated according to the most rigid diagnostic criteria reveals a quite typical picture: clinically very early onset of infections and death within the first months of life: the infections involve the skin, the respiratory and the gastro-intestinal tract. Thrush infection is quite common. Laboratory investigations show consistent lymphocytopenia and dysfunction of the lymphocytes as the base of the cel lular defect along with agammaglobulinemia (humoral deficiency). The basic pathology findings are a dysplasia of the lymphatic system and especially of the thymus. Genetically the disease is different from Bruton's agammaglobulinemia, since many families have been observed in which an autosomal recessive transmission has to be assumed however a second way of sex-linked transmission seems to occur as well.

Finally a third type of pure alymphocytosis has been observed by Nézelof presenting with exactly the same thymus pathology as the combined cases of the Swiss type. However the immunoglobulin concentrations are normal.

Hypothesis. In animal experiments the basic influence of the thymus upon the development f the lymphatic system in rodents and of the bursa Fabrici upon the development of humoral immune mechanisms in fowl have been described. Although the uncritical analogy of these animal models with man is certaly dangerous, i is tempt ing to make the following pecul tions. the cases observed by Bruton might be the esult of a deficiency of the equ alent of the bursa Fabrici for the patients described by Nézelof an early dysplasia of the thymus mi ght give an explanation, and in the Swiss type of agammaglobulinemia both factors might be ope at ve at the same time

Therapy until now has been completely unsuccessful. The early replacement of the missing organs, a thymus and the equivalent of the bursa has been tried. Some authors f lt that fetal organ should be used to void a graft versus-host eaction. However until now no improvement whatsoever has been achieved.

Herbert C. Miller (Kansas City USA): *Prematurity and respiratory distress syndrome*

Gestational age and sex are important factors in the pathogenesis of RDS. Birth weight and calculated gestational age together are inadequate for defining true gestational age in about 25% of infants born in our clinic and weighing less than 2500 g. True gestational age can be more accurately defined by including measurements of bone age, body length and head circumference plus an assessment of the infant's neurological development.

Male negro infants with birth weights from 1500 to 2500 g and having calculated gestational ages between 30 and 38 weeks had significantly higher incidence of RDS than female negro infants of comparable weights and dates, the incidence among males being 2 to 4 times higher than among females. The female apparently matures in some way in utero more rapidly than the male. Birth weight is not by itself a reliable indication of maturity.

Meeting Oct. 4 1967

Ingo Hellmers (Bremen): *Health education in the Rudolf Steiner schools*

Meeting Oct. 28 1967

John Lind (Stockholm): *Placenta transfusion and the physiological adaptation*

Meeting Dec. 9 1967

Sulo Toivonen. *The normal development of the fetus*

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PROCEEDINGS OF PEDIATRIC SOCIETIES

SCANDINAVIAN ASSOCIATION OF PAEDIATRIC SURGEONS

Meeting Sept. 29 – Oct. 1 1967

M. Palmén. *Malignant tumors of children*

256 cases of malignant tumors treated in Helsinki University Children's Hospital, 1946–1966, are reviewed as to frequency age distribution, and operability Brain tumors and leukaemias are excluded. The cases are grouped as follows.

Nephroblastomas (100 cases) are published by Sirola.

Neuroblastomas (57) occurred mostly below 7 years of age. 9 were operated upon radically 4 inoperable cases have survived 3–7 years.

Osteogenic sarcoma (17) occurred mostly over 7 years of age and had very poor prognosis, whether operated upon or not.

Fibro-lipo-myosarcoma of soft tissues (18) had an even age distribution between 0–15 years. Radical surgery has given favourable results.

Ovarian malignant tumors (9) occurred only above 7 years of age Most have died.

Testicular malignant tumors (5) were all below 2 years of age and are uniformly fatal.

Sarcoma botryoides (11) occurred in all age groups. Two have survived over 3 years after radical cystectomy

Sacrococcygeal malignant teratoma (9) occurred below 2 years f age. All have had recurrence. 33 benign sacrococcygeal teratomas have been treated during the same period.

Of other tumors (39) 2 were localized to digestory canal, 8 to adrenals, and 16 to lymphatic tissue.

Kari Sirola. *The treatment of Wilms' tumour and the results*

A total of 102 cases of Wilms tumour were seen at the Children's Hospital of Helsinki University 1947–67 Of this material 35 patients (34 per cent) are living. In radical operations (45 cases) the survival was 58 per cent, in subradical operations (43 cases) only 21 per cent. The prognosis is better in young children than in the older During the years the results have become better This is considered to be due, not so much to the development of surgery and anaesthesia, but to the more effective radiotherapy and, in the last years, the cytostatic therapy The improvement in the results is observed especially in the group of older children, and on the other hand in the group of subradically operated patients. Since 1963 cytostatic drugs—mostly Actinomycin D—have been systematically used combined with the operation and postoperative irradiation. Although the time of observation in this last series still is relatively short the results in operative cases seem to become better In these cases, we may nowadays expect a survival of about 60 per cent. Four cases of bilateral tumours are included in the material two of these patients have survived more than one and a half years. In three cases a lobectomy for pulmonary metastasis was performed, and all these patients are alive. As for complications, the children seem to be relatively resistant for cytostatic and irradiation therapy

Olof Lindfors *Ovarian tumours in children 0–14 years of age A preliminary report of 71 new cases from Finland and Sweden*

71 new cases of ovarian neoplasms in girl 0–14 years of ge are reported. The material is collected f om the period 1948–1966.

Ovarian tumours are rare in childhood, three cases per one million inhabitants every year 1 in

..00 patients admitted to a pediatric surgery unit is suffering from an ovarian tumour

30 per cent are malignant. The younger the patient is, the higher is the malignancy rate Th morbidity rate increases with the age.

The duration of symptoms before admittance is varying and a little bit longer in Finland than in Sweden but most patients are admitted within one month.

Main symptoms (twisted cases excluded) causing the first consultation are pain and a large abdomen. The growth is sometimes very fast.

Sedimentation rate is increased in most malignant cases. Increased white cell count is found only combined with torsion in the acute phase. Calcium of dermoid cysts can be seen in X-ray pictures. The size varies from mandarine to football size. The most common benign tumour is the dermoid cyst and among the malignant n the dysgerminoma and teratoma dominating. 20% were twisted. All cases were operated on and one died during the operation. It has been difficult to follow up the cases. In 16 cases the time of death was known. 80% of the malignant cases dye within 7–8 months whether they are given radiation and/or cytostatic therapy or not.

If metastases are found at the primary operation the prognosis is extremely bad.

N O Ericsson & O Qvist: *Testicular tumors in childhood*

Primary testicular tumors are rare. Only small series ha e been published. In ?0 years we have seen .. patients, aged 6 months to 11 years.

Benign tumors were present in 9· 8 teratomas and 1 lipoma. Malignant tumors were found in 13 7 carcinomas, 3 sarcomas, 2 malignant teratomas and one metastatic neuroblastoma No seminom or endocrine tumor was found. All tumors occurred in descended testicles.

Clinical signs and age did not permit preoperative evaluation of malignancy in this small group.

Treatment: Excision of testis, epididymis and part of the cord in all, postoperative irradiation in 5 actinomycin in 3 no retroperitoneal gland dissection.

Results. Benign. all alive. Malignant: 7/12 primary tumors alive, 3/7 carcinomas, 3/3 sarcomas. Postoperative observation time 6–20 years. Remaining 5 malignant died from tumor including all 3 with actinomycin treatment.

A great number of tumors were benign. The prognosis for the malignant group was about the same as in the literature, 50% surviving.

Aarne E. Rintala. *Malignant melanoma in a 6-year-old boy*

A malignant melanoma of the skin in children is a rarity At least 30 sufficiently documented cases have been published in the literature. One case, treated at the Helsinki University Children's Hospital, is added.

A boy aged 6 years, developed a malignant melanoma in a wide pigmented nevus on the parietal region of the scalp with metastases in the regional neck glands at the time of primary diagnosis, radical neck dissection, irradiation and cytostatic treatment, pulmonary metastases developed and the patient died one year after the initial treatment.

Only three of the reported 30 patients have been alive and cured after an observation period of five years. The benign juvenile melanoma is still commonly mistaken for a malignant one and the importance of early differential diagnosis is stressed.

G. R Wallgren. *Burkitt's lymphoma*

In addition to a general survey a case observed at the Aurora Hospital, Helsinki, was presented.

A 4-year-old girl with a firm tumour in the left maxillary region was admitted to the hospital in October 1966 A biopsy indicated lymphoma. Atypical vacuolated cells were seen in bone-marrow smears. The peripheral blood picture never indicated leukaemia. The serum gave a positive reaction with cell cultures from Burkitt's lymphoma in the membrane immunofluorescence reaction. Treatment with methotrexate and other drugs was unsuccesful. The child died in January 1967

At autopsy the maxillary tumour infiltrated in the left middle cranial fossa. Nodes of tumour were present in various parts including the parietal pericardium and the ovaries. The girl had never been in Africa or elsewhere outside Finland.

E. Schaefer *A case of adrenocortical carcinoma successfully treated by surgery*

A case of adrenocortical carcinoma in an 18-month-old baby is reported. The clinical features were clearly defined Cushing's syndrome with moon face, obesity of the trunk, asthenia, hypertension, polycythaemia and reduction in the fasting granulocyte count, radiological osteoporosis. Adrenogenital syndrome in a mild form. Greatly elevated serum and urinary cortisone and 17 ketosteroid levels. Intravenous pyelogram revealed displacement of the upper left pole of the kidney by a well-defined tumor the size of a golf ball.

The tumor was extirpated *in toto* together with the left kidney through a large abdominal incision. Histologically the tumor was an adrenocortical carcinoma. No metastases could be detected. In the 9 months postoperative observation period the clinical picture and the cortisone and steroid values returned to normal, the patient's state of health was very good.

The report draws attention to the advantages of the transperitoneal operative route via an incision at the edge of the costal arch. The dosage schedule proposed by Wilkins for pre- intra- and postoperative cortisone substitution therapy proved highly valuable. Statistical evaluation of cases reported in the literature is hardly possible owing to the great difficulty in differentiating pathologically between carcinoma and hyperplasia of the adrenal cortex.

A. Aperia, U. Berg, O. Broberger & N. O. Ericsson. *Renal function in dysplastic kidneys*

It is generally assumed that small kidneys are the result of secondary shrinkage due to chronic pyelonephritis, that they are irreversibly destroyed and should be removed. Small kidneys can, however, also be due to congenital underdevelopment of the renal parenchyma, to renal dysplasia. The latter means, theoretically, that a reduced number of nephrons are formed, which may function normally.

20 patients, aged 3–17 years, with renal dysplasia became subjects of renal function studies, one part of the work dealing with separate investigations of the patients' two kidneys, another part with determination of tubular function particularly.

The investigation revealed that:

- the renal function as measured by glomerular filtration rate and tubular transport capacity cannot be adequately predicted by intravenous urography;
- dysplastic kidneys have a reduced number of functioning nephrons and that these are overperfused;
- there is a disturbance in Na-reabsorption and concentration capacity with a linear correlation between these two parameters;
- kidneys with a more pronounced damage to the sodium reabsorption have a highly significant increase in glomerular filtration rate and renal plasma flow during water diuresis.

The last finding brings a therapeutic implication of this study, namely a theoretical basis for the old empirical observation that forcing fluid into patients with pyelonephritis is generally beneficial.

Another clinical implication brings the finding that renal function in dysplasia is often much better than that suggested by the roentgen films. Thus, a patient with renal dysplasia should not be nephrectomized until detailed renal function studies have been performed.

Jan Johannesen. *Function of the hydronephrotic kidney*

It is generally accepted that hydronephrosis is caused by an increase in the intrapelvic pressure. Acute obstructions always produce a raised pressure, but normal intrapelvic pressure is frequently observed in the chronic progression of hydronephrosis.

Micro-dissection and studies with electronmicroscope have demonstrated atrophy of the cells in the proximal tubules and in the collecting ducts. This fact does not agree with the functional disturbances. Thus the maximal re-absorption of glucose has not decreased during the early development of hydronephrosis.

Experimental studies demonstrate a marked decrease in the renal blood flow. The arterio-venous oxygen difference is unchanged, but the total consumption of oxygen is decreased in proportion to renal blood flow.

Glomerular filtration rate is reduced in hydronephrosis, but the filtration continues even in

total obstruction. Thus the hydronephrotic pool is not a static unchangeable solution. Experimental results suggest both a pyelo-venous and a pyelo-lymphatic drainage from the dilated pelvis.

The renal concentrating ability is markedly impaired in hydronephrosis and there is a decreased concentration gradient from cortex to inner medulla. For this reason, polyuria may be an early symptom in hydronephrosis. The acidifying capacity of the hydronephrotic kidney is also reduced.

It is not proved whether nephron destruction is caused by mechanical pressure against the tubular cells or by a decreased oxygen supply

The drainage of hydronephrosis is frequently followed by an enormous loss of salts and water indicating a marked impairement of tubular salt reabsorption. This may be disastrous without adequate intravenous substitution.

N O Ericsson & U Rudhe *Prognosis of non obstructive vesico-ureteral reflux*

Several antireflux operations have been published but the indications for surgery have been little discussed. Although reflux is abnormal it should not necessarily be considered permanent or dangerous.

In an attempt to study the evolution of reflux following medical treatment we studied 710 reflux patients. Of these 231 (33 %) were excluded because they had various types of urinary obstruction. The remaining 479 had non-obstructive infections with reflux 170 became subjects of detailed studies. However conclusions should be drawn with care because of the errors involved in reflux diagnosis.

The incidence of reflux in non-obstructive infections was 35 % in both sexes. Girls constituted $^2/_3$ of the series. Hydronephrosis was demonstrated in 22 % small or incipient in further 27 %

At follow-up study reflux was no longer demonstrable in 33 % in 24 % it was of lesser degree, only 8 % demonstrated increased reflux. Even massive reflux disappeared in 25 % of that group. The reflux prognosis was the same in both sexes and the various age groups. There was a definite correlation between infection and reflux when reflux disappeared the infection was cured or controlled in 100 % when reflux persisted unchanged the infection was cured in 50 %

Hydronephrosis diminished in 33 % increased in 20 % although markedly so only in a few No patient with normal urography at the first examination developed hydronephrosis in the follow-up period.

Thus, reflux may disappear or improve in more than 50 % of the cases following only medical treatment of infection and without resort of operation. In 10–20 % reflux may constitute a risk, sometimes grave.

O Knutrud *Ureteric duplications treated with heminephrectomy*

At the Children s Clinic of the Rikshospital in Oslo 188 ureteric duplications was found among 5941 children examined with i v p. Of these were 75 children admitted to the clinic for further examination and treatment. 71 of them had recurrent urinary infection with up to 10 years duration before admission.

Conservative treatment 4 pat. examined for enuresis only—no hydronephrosis.

14 pat. with infection, but without hydronephrosis have all been free of symptoms after 6 months medically treatment.

4 pat with moderate hydronephrosis—3 of them later operated upon due to recurrence of infection

2 pat. bilateral duplication with hydronephrosis in all four parts, but no reflux or stenosis.

Surgical treatment in 51 patients 35 pat. heminefrectomy and ureterectomy—31 of these completely free of symptoms.

4 pat. nefrectomy and free of symptoms.

6 pat. with incomplete duplication treated with reimplantation of ureter—they are all free of reflux.

3 pat. cutaneos ureterostomy due to neurogenic bladder and/or uremia. They are all better

2 pat. treated with uretero-uretero-anastomosis with poor result.

1 pat. pyelo-uretero-plasty and ureterectomy with a good result.

We prefer the heminefrectomy because the involved part of the kidney usually is affected with heavy pyelonefritic scarring.

Discussion

H. Sommerschild. Comments on the pyelo-ureteric anastomosis:

Bifid ureter occurs in 30% of ureter duplications. 75% confluates in the distal third which predispose to infection. Possible reflux will occur in both branches. In these patients there is only diffuse—and often moderate—changes of the kidney. Nephrectomy does not come into question and it is difficult to decide which part to resect by heminephrectomy.

The anastomosis between pelvis/ureter and pelvis/ureter with resection of one of the ureter branches is then a good, kidney conserving, solution—removing the possible cause of the infections.

The method is applicable even to other types of ureter duplication when the "kidney situation" would be the same.

J. Cendron & H. Saied (*J. d'Urologie et de Nephrol.* 1966) have since 1957 operated 24 children in this way with very promising results. Their patients are followed 1–3 years.

N. Genton (*Kinderchirurgie* Suppl. *101/114* 1966) has used this procedure on 11 of his patients with very good short time results.

We have made 6 such anastomosis in the last 3 years—in 4 conserving the lower ureter, in 2 the upper one. ½–3 years after operation all have free passage through the anastomosis without any signs of pelvic dilatation, without reflux and without bacteruria. Only one patient has yet discontinued chemotherapy, one is on the point of trying and in the other 4 it is still too early to finish the chemotherapy.

E. Normann: *Surgical treatment of pyloric stenosis*

A presentation of 100 operated cases of congenital pyloric stenosis, treated at Ullevål Hospital, Oslo, in a three-year period from 1964 to 1967 is given. Previously only about 50% of the patients with this disease were operated upon, the other half were treated medically.

There has in this material been no mortality and no serious complications.

The hospitalization time averaged 10 days, 3.5 days preoperatively and 6.5 days postop.

These results compare favourably with the best results obtained from medical treatment. We have then come to the conclusion that congenital pyloric stenosis is a disease that should be treated surgically.

Th. Ehrenpreis, K. A. Norberg & C. Wirsén. *Sympathetic innervation of the colon in Hirschsprung's disease. A histochemical stud*

Rectosigmoidectomy specimens from 10 patients with Hirschsprung's disease were studied histochemically by a fluorescence technique permitting selective visualization of adrenergic nerve terminals. In histologically normal segments of bowel, synaptic arborizations around the intrinsic ganglia were present and had the same appearance as demonstrated earlier in several animal species. In aganglionic bowel no adrenergic synapses were found.

These findings imply that the mediation of not only parasympathetic but also sympathetic influences to aganglionic bowel is interrupted. Functional denervation promotes spastic contraction, which is the main characteristic of the distal aganglionic segment of bowel in Hirschsprung disease.

G. R. Wallgren. *Langenskiöld's operation for large ventral hernias*

The operative method for repair of large incisional herniae was introduced by F. Langenskiöld (*Acta Chir Scand.* 1944). At the Aurora Hospital, Helsinki a modification of the original method has been used in four infants with very large ventral herniae. In all four a primary operation by skin covering had been performed at birth, in two for omphalocele and in two for gastroschisis. In all cases a very favorable final result was achieved.

C. Lohr, L. Okmian & O. Ekloef: *Pseudodiverticula of the esophagus in 2 newborn babies*

In two newborn babies with symtoms simulating esophageal atresia X-ray-studies visualized what appeared to be a posterior diverticulum of the upper esophagus. The first baby was operated upon 1 days after admission. At operation no

diverticulum and no other pathology was found. Repeated X-ray-studies 5 days postop. showed a normal esophagus. With this experience in mind the second baby was treated conservatively with nasogastric feeding tube for 14 days. X-ray investigation at this time showed a normal esophagus. Only a few cases of true diverticula in children have been reported. X-ray findings similar to those seen in our two cases have been reported in one adult patient after instrumental perforation of the esophagus. The postoperative course was also similar. This supports the theory that the pseudodiverticula in our patients were caused accidentally during pharyngeal suction in the delivery-room. Accordingly perforation of the mucous membrane resulted in a via falsa between the two layers of the esophageal wall. The fact that they healed spontaneously excludes a congenital etiology. In conclusion the importance of soft catheters and a traumatic suction-technique in newborn babies is stressed.

B. Björck, B. Ivemark, A. Livaditis & L. Okmian. *Esophageal anastomosis. Experimental studies in newborn pigs*

Leakage and stricture following anastomosis for esophageal atresia are still common and serious complications. This problem prompted us to perform an experimental study on the healing and function of various new types of esophageal anastomosis. In addition the role played by the muscular and mucosal layers in stricture formation was selectively studied.

The material consisted of 21 pigs of 3–4 kg of body weight. In 16 animals division of the esophagus and anastomosis either by means of circular clamp or silk sutures was carried out. Both in suture and clamp anastomosis the entire esophageal wall or only the mucosa was selectively used. In the remaining 5 pigs circular division or removal of the muscular layers only was performed.

The results were evaluated on the basis of cineradiographic, gross and microscopic examinations. The width of the anastomoses was assessed under standardized conditions. The experience gained from the present series indicates that the sutureless technique results in a tight anastomosis. There was no difference in the incidence of stricture formation between the groups of clamp and suture anastomosis. The high frequency of strictures in clamp anastomosis could be related to tissue damage due to difficulties in the application of the clamp. An improved clamp construction has eliminated these problems. A contributory factor in the development of stricture is the fibromatous response to tissue damage in the pig esophagus. This also serves to explain the results in suture anastomosis.

BOOK REVIEWS

G. Wolstenholme & M. O'Connor (eds) *Health of Mankind* (Ciba Foundation 100th Symposium), 297 pp. J. & A. Churchill Ltd London 1967 60s.

The 100th Ciba Symposium was held in March 1967 and devoted to three aspects of "the health of the whole world": the assessment of the present health of mankind, major factors aggravating world health problems and the resources of manpower for solving the problems.

Amongst the participants were Dr M. G. Candau, Director-General of the World Health Organization, and Dr G. Pincus, well-known pioneer in the field of hormonal birth control.

Dr Pincus died a few months later. The book has been dedicated to his memory.

Any assessment on a global scale of health problems requires extrapolations and that part of the book can best be summarized by quoting Dr Candau's comment on one of the papers: "You are to be congratulated on having the courage to present those world figures. No new facts leading to solutions or strategic planning can hardly be expected to come out of such data and yet in order to get some idea about what we are up against such analyses are necessary."

Dr Pincus illustrates the growth of the population of the world by computing out the following relative figures: Asia's proportion will rise from 55 per cent in 1950 to 60 per cent in 2000, Latin America's from 6.5 per cent to over 9 per cent, Africa will remain constant at 8 per cent, Europe will drop from 23 to 15 per cent and North America from 7 to 5.

In order to provide in 2000 the incomes which existed in Europe in 1950, Asia will have to increase the income per head 42-fold!

The most urgent needs appear to be those of India. Food production cannot keep pace but it could run slightly better chance if as much as one third were not destroyed. ... war against rats should be declared.

Food production is intimately connected with water supplies—in South Asia 61 per cent of the urban population is not served from public supplies. In contrast 86 per cent in Latin America has such facilities according to Professor Wolman.

Dr Evang, head of the Norwegian Health Services describes the limiting factors in health control. Dr Evang is one of the three midwives of the WHO with an interesting story about its birth and difficulties and why it has not been able to play a more active rôle.

Only manpower can solve the present and future problems. The third part of the book reviews education and training. Who wants to be a doctor and are the students being selected in a useful way? No country has yet been able to forecast correctly the needs of doctors and no physician could have foreseen the particular course he took in his professional life.

A wide basis of recruitment is required but not by free admittance and later failure. An attractive suggestion to be added to present methods is promotion from the paramedical professions (nurses, feldshers, medical assistants).

The disappearance of the general practitioner and how to bring him back is touched upon. Is the lack not simply because we seek less and less to train him? Better training in his own field leading to the speciality of general practice appears to be the only remedy.

Training of doctors requires clinical research. Pequignot of Paris and Banks of Cambridge suggest for the financing of clinical research that whenever payment is made for a medical service or to the doctor something should be included for research just as the price of a drug includes financing the research leading to its discovery. This is a sort of philosophy which administrators of health services should be told.

The symposium ends with the presentation by Dr Wolstenholme of a plan for a World Health Service—an organization to be mobilized where and whenever needed. Everybody present appears to have been taken by surprise. Dr Candau asked for a little time to think. There could be little choice but to applaud the suggestion presented by the host of the occasion.

In the reviewer's opinion the brilliant record of the Ciba Foundation has hardly been given the monument earned at for its 100th symposium by this volume. But it was not an easy task.

Åke Nordén

Jørgen Pedersen. *The Pregnant Diabetic and her Newborn*. Munksgaard, Copenhagen 1966. Dan. kr. 60.

The introduction of insulin treatment radically changed the prognosis of juvenile diabetes but was also followed by new medical problems. One of increasing importance has been the management of the pregnant diabetic woman. During the pre-insulin era few diabetic women conceived. To-day the young diabetic girl does not only survive into fertile age and in many cases lives through the whole period of child-bearing but her fertility has also increased. The result has been an increasing number of diabetic pregnancies and there are nowadays few obstetrical and paediatric departments escaping the problem how to manage the pregnant diabetic and her newborn. As about one delivery out of 1000 concerns a diabetic mother it is evident that real experience of this condition can be obtained only at larger centres and a summary of our present knowledge with directions for treatment of the pregnant diabetic and the newborn is for that reason very welcome. Jørgen Pedersen's monograph is not only a manual for management of diabetes in

pregnancy founded on twenty years f personal experience and a profound knowledge of the literature but also an introduction to and a careful evaluation of the new problems in this field of diabetology It is significant that the last chapter is devoted t the "Screening for actual or subsequent diabetes in pregnancy" It is clear from Pedersen's book that there still are many controversial points and unsolved problems concerning the pregnant diabetic but one thing seems to be unquestionable. The management f this condition is a difficult task which demands teamwork in order to obtain good results. Internists, obstetricians and paediatricians have to cooperate and the paediatrician notes with satisfaction the author's opinion that "a prophylactic battle against the neonatal complications should be fought during pregnancy This book is recommended not only to those directly concerned with the management of the pregnant diabetic but to all who take an interest in the problems of diabetes.

C O Bergstrand

Wilhelm Sinner *Röntgenatlas der Erkrankungen der oberen H rnwege bei Säuglingen und Kleinkindern* 132 pp Illus. VEB Gusta Fischer Verlag, Jena 1966 Sw kr 95

This book presents material of infants and children examined and treated at the urological department, University of Rostock (DDR) The foreword does not clearly indicate for whom the book is intended It seems, however, quite reasonable to believe that it is written mainly for urologists performing their own radiology

The author discusses clinical and radiological aspects of urological disease in 76 cases. He apparently prefers trograde to intravenous pyelography which contrasts with standard practice in Scandinavian pediatric urological and radiological centers. This is quite understandable as the advantages of modern I.V.P-techniques seem to be unknown to him The use of additional projections including true lateral views, as well as the use f voiding urethrocystography would have been rather helpful in evaluation of the radiological findings presented

From radiological point of view the book lacks interest and its value to the urologist is questionable

Ole Eklöf

H. J Kaufmann (ed.) *Progress in Pediatric Radiology* volume I *Respiratory Tract* 354 pp., Illus. S. Karger AG, Basel and New York 1967 Sw kr 100

This volume is the first in series intended to give radiologists and pediatricians recent information and critical appraisal of present thinking and approach in selected fields of pediatric radiology By invitation of the editorial board, number of authors, mainly pediatric radiologists, discuss different radiological aspects of respiratory tract disease in infants and children. Emphasis is placed on examination of the lungs and thoracic cage.

The typography is mainly of high standard. The figures and legends, howeve are rather unsuitably placed making several articles difficult to read. Space is unnecessarily wasted by inadequate trimming of survey films. Detailed views of areas of interest would have been appreciated. It is hoped that the editor will, in subsequent volumes, select the most informative illustrations. There is no need in such publication to demonstrate metallic foreign bodies in the lungs nor for repetative illustrations of the same disease. The use of double columns would ha e provided more space.

As stated in the foreword the opinions expressed in the book are entirely those of the authors. This gives the reader an interesting iew of radiological thinking and methods in medical centers throughout the world. The discussion of the special treatment article is very informative. Editorial comments reflecting the collective knowledge and xperience of the members of the editorial board would have been valuable addition to many of the subjects presented. This could have been done without increasing in the size of the volume if fewer topics were discussed.

Apart from this mostly formal criticism, it is the reviewer' opinion that this first volume of *Progress in Pediatric Radiology* will prove quite successful. It fills a gap in pediatric radiological literature and should be recommended both to radiologists working in this field and to pediatricians interested in radiology

Ole Eklöf

Erna Christensen & Johannes Melchior *Cerebral Palsy—A Clinical and Neuropathological Study* (*Clinics in Developmental Medicine N .5*), 127 pp. W Heinemann Medical Books Ltd., London 1967 28s

In this book an attempt is made to find the anatomical lesion in the brain corresponding to the clinical syndromes in cerebral palsy This is no doubt difficult task. The *material consists of 69 patients with cerebral palsy* clinically diagnosed after standard principles, who died before 15 years of age. All the patients had extensive pathology which made the correlation more difficult. The authors give detailed presentation of the clinical pictures and the neuropathological techniques and findings. The neuropathological findings are grouped in two different ways, compared on one hand with the clinical picture and on the other hand with the supposed aetiology Detailed reviews are given in the most interesting cases. This study contributes to the understanding of cerebral palsy and one might only wish with the authors that, in the future, cases of cerebral palsy of mino degree should also be described in the same way

Ingrid Bjerre

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IMMUNOGLOBULIN LEVELS IN INFANTS WITH LOW BIRTH WEIGHTS

Torsten Berg

From the Department of Pediatrics and the Blood Centre, University Hospital, Uppsala, Sweden

Premature infants, especially those with low birth weights, have a higher morbidity and mortality from infections than full-term infants. This is particularly true of the very first few weeks of life but also holds for the later part of the first year of life. For this reason attempts have been made to reduce the frequency and severity of these infections by prophylactic treatment with gamma globulin. Steen (13), using relatively small doses of gamma globulin, found no positive effect of such treatment. Amer *et al.* (1) presented in 1963 the results of a double-blind study where from a large number of prematurely born infants a group selected at random received large doses of gamma globulin administered at monthly intervals. They found that a significantly larger number of these infants had no infections, and fewer had mild infections, during the first year of life. Deaths from infections showed a similar trend in favour of the gamma globulin group but this difference on its own was not statistically significant. No systematic classification of the infants according to birth weight and gestational age was reported.

In 1952 Norton *et al.* (11) showed, by means of electrophoresis, that prematurely born infants had, on the average, lower gamma globulin levels than full-term infants. Bergstrand & Czar (3) showed in 1957 that in foetuses 9–19 weeks old the amounts of gamma globulin increased with length of gestation. Haworth and co-workers (4) published in 1965 a study of the different immunoglobulins in 19 premature infants from whom samples had been taken at birth and subsequently at monthly intervals during the first year of life. The size of the series did not permit systematic study of the immunoglobulin levels in relation to birth weight and gestational age. The levels of γG globulin at birth approached the levels found in normal adult serum. No statistically significant correlation was found between the serum concentration of the immunoglobulins and the incidence of infection.

The following describes a study of the immunoglobulin levels at birth and during the first few weeks of life in infants with low birth weights. A preliminary report on the results in 47 of the infants in this series has been given previously (2).

MATERIAL AND METHODS

The material consisted of 65 infants with birth weights lower than 2500 g, treated at the Department of Pediatrics, University Hospital, Uppsala from spring 1966 to autumn 1967. As from the spring of 1967 only children with birth weights lower than 1800 g were included in the study. The IgG levels are also reported for some non-viable foetuses with gestational ages varying between 16 and 26 weeks.

The blood samples were taken, as a rule during the first day of life and at the ages of three and seven days, and subsequently at intervals of one or two weeks for the remaining hospitalization period. From some infants additional samples were obtained some time after their discharge from hospital. In some infants only few isolated samples were obtained, due largely to the high mortality in the smallest infants or to the short stay in hospital of the larger ones.

On few occasions samples were taken one or two days before or after the time originally intended. When considered suitable the results of the analyses of these samples have been included in appropriate groups for the statistical calculations. Some IgG values were thus accepted if the samples were taken at an age of four days instead of three days, or at an age of five, six or nine days instead of seven days, provided that the average age of the group was not changed notably. On the same conditions some IgM values were accepted if the samples were taken from infants at an age of four instead of three days, or six instead of seven days. The great majority of samples, however, were taken at the time intended.

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pregnancy founded on twenty years of personal experience and a profound knowledge of the literature but also an introduction to and a careful evaluation of the new problems in this field f diabetology It is significant that the last chapter is devoted to the "Screening for actual or subsequent diabetes in pregnancy It is clear from Pedersen book that there still are many controversial points and unsolved problems concerning the pregnant diabetic but one thing seems to be unquestionable. The management of this condition is a difficult task which demands teamwork in order t obtain good results. Internists, obstetricians and paediatricians have to cooperate and the paediatrician notes with satisfaction the author opinion that "a prophylactic battle against the neonatal complications should be fought during pregnancy This book is recommended not only to those directly concerned with the management of the pregnant diabetic but to all who take an interest in the problems of diabetes.

C G Bergst and

Wilhelm Sauer: *Röntgenatlas der Erkrankungen der oberen Harnwege bei Säuglingen und Kleinkindern* 132 pp Illus. VEB Gustav Fischer Verlag, Jena 1966. Sw kr 95

This book presents material of infants and children examined and treated at the urological department, University of Rostock (DDR) The foreword does not clearly indicate for whom the book is intended. It seems, however quit reasonable to belie that it is written mainly for urologists performing their own radiology

The author discusses clinical and radiological aspects f urological disease in 76 cases. He apparently prefers retrograde to intravenous pyelography which contrasts with standard practice in Scandinavian pediatric urological and radiological centers. This is quite understandable as the advantages of modern I.V.P.-techniques seem to be unknow to him The use of additional projections including true lateral views, as well as the use of oiding urethrocystography would hav been rather helpful in evaluation of the radiological findings presented.

From radiological point of view the book lacks interest and its value to the urologist is questionable

Ole Eklof

H J. Kaufmann (ed). *Progress in Pediatric Radiology* olume I. *Respiratory Tract* 354 pp Illus. S. Karger AG, Basel and New York 1967 Sw kr 100.

This volume is the first in series intended to give radiologists and pediatricians recent information and critical appraisal of present thinking and approach in selected fields of pediatric radiology By invitation f the editorial board, number of authors, mainly pediatric radiologists, discuss different radiological aspects of respiratory tract disease in infants and children. Emphasis is placed on examination f the lu gs and thoracic cage.

The typography is mainly f high standard. The figures and legends, however are rather unsuitably placed making several articles difficult to read. Space is unnecessarily wasted by inadequat trimming of survey films. Detailed views of areas of interest would have been appreciated. It is hoped that the editor will, in subsequent olumes, select the most informative illustrations. There is no need in such publication to demonstrate metallic foreign bodies in the lungs nor for repetitive illustrations f the same disease. The use f double columns would have provided more space.

As stated in the foreword the opinions expressed in the book are entirely those of the authors. This gives the reader an interesting view of radiological thinking and methods in medical centers throughout the orld. The discussion f the special treatment article is very informative. Editorial comments reflecting the collective knowledge and experience of the members of the editorial board would have been valuable addition to many of the subjects presented. This could have been done without increasing in the size of the volume if fewer topics were discussed.

Apart from this mostly formal criticism, it is the reviewer s opinion that this first volume of *Progress in Pediatric Radiology* will prove quite successful. It fills a gap in pediatric radiological literature and should be recommended both to radiologists w rking in this field and to pediatricians interested in radiology

Ole Eklöf

Erna Christensen & Johannes Melchior: *Cerebral Palsy—A Clinical and Neuropathological Study* (*Clinics in Developmental Medicine N 25*), 127 pp. W Heinemann Medical Books Ltd London 1967 28s.

In this book an attempt is made to find the anatomical lesion in the brain corresponding to the clinical syndromes in cerebral palsy This is no doubt difficult task. The material consists of 69 patients with cerebral palsy clinically diagnosed after standard principles, ho died before 15 years f age. All the patients had extensive pathology which made the correlation more difficult. The authors give detailed presentation of the clinical pictures and the neuropathological techniques and findings. The neuropathological findings are grouped in tw different ways, compared on one hand ith the clinical picture and on the other hand with the supposed aetiology Detailed reviews are given in the most interesting cases. This study contributes to the understanding of cerebral palsy and one might only wish with the authors that, in the future, cases of cerebral palsy of minor degree should also be described in the same way

Ingrid Bjerre

Table 2. *Initial IgG levels in mg/100 ml*

The infants are grouped according to birth weight

Age, days	0–3		
	No	Mean	s.
Birth-weight, g			
< 1500	14	525 (249–771)	139
1500–2000	21	860 (315–1446)	279
2001 2500	30	957 (444–1593)	294

tional age in Table 1 and Fig. 1 and with birth weight in Table 2. The initial IgG concentration shows good correlation with gestational age. The mean value in group 2 with an average gestational age of 33.6 weeks differs significantly from that in group 3 with an average gestational age of 37 weeks ($p < 0.01$) Similarly the mean value in group 3 differs significantly from that in umbilical cord sera from group of full-term infants ($p <$ 0 025). The mean value in group 1 with an average gestational ge of 30 weeks does not differ from that in group 2 in a quite significant way ($p \lesssim$ 0.05) On the other hand, there is no t tistically significant difference between the init l IgG concentrations in infants of the weight gro ps 1500–2000 g and 2001–2500 g.

The subsequent development of the IgG concentrations is shown in Table 1 and Figs. –3 The IgG levels seem to fall at about the same rate in the three groups. As is most evident in Fig. 3 where the development of IgG levels in individual infants is given, children of low gestational age, in particular run a great risk of getting very low IgG concentrations already in the course of the first weeks of life. For instance one child of a gestational age of .8 weeks had an IgG concentration of 120 mg/100 ml at six weeks of age.

IgM The development of IgM concentrations is shown in Table 3 and Figs. 4–5 The initial levels do not differ noteworthily from those found in umbilical cord sera of full-term infants (7) There is no marked change in IgM concentration during the course of the first day of life but subsequently there is a pronounced increase This increase is obvious in almost every individual infant in all groups as early as at the age of 3 days. In the group with the lowest gestational age (only 3 infants available for comparison) the individual infant show an average rise in IgM concentration

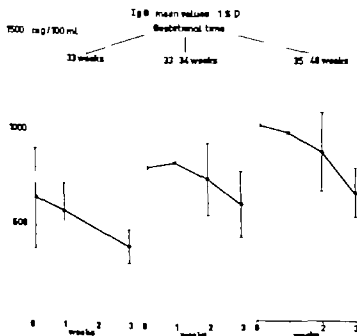


Fig. 2 Average IgG concentrations ± S.D. p to the age of 3 weeks The infants are grouped according to gestational age.

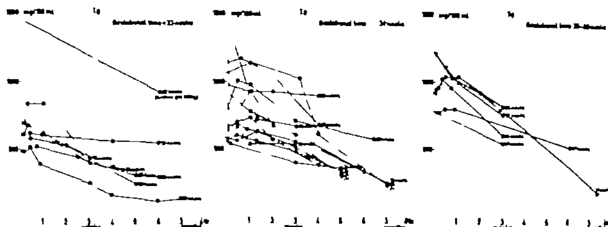


Fig 3 The development of IgG concentrations in individual infants, grouped according to gestational age. The infants were followed during at least the first 3 weeks f life.

of 5 7 mg/100 ml from the age of 0–1 days to the age of 3 days. In the group with a gestational age of 33–34 weeks (13 infants are compared) the IgM rises, on the average, by 2.9 mg/100 ml up to the age of 3 days, and in the group with a gestational age of 35–40 weeks (11 infants compared) the average increase is 4 0 mg/100 ml. In all groups the increase is statistically significant ($p<0\ 01$ <0.025 resp $<0\ 005$)

In all three groups the IgM continues to rise up to the age of three weeks, but this increase is considerably less pronounced, however in the group with the lowest gestational age than in the other two groups. Thus at the age of three weeks the infants in group 3 have an average IgM concentration of 44.5 mg/100 ml whereas those in group 1 exhibit, on the average, only half that concentration, i.e. 21 7 mg/100 ml.

IgA The majority of the infants in this series had no demonstrable IgA until the age of 3 weeks

Table 3 *IgM in mg/100 ml (average levels, range and* S.D)
The infants are grouped according to gestational age

Age, days	0			1			3		
	No.	Mean	S.D.	No.	Mean	S.D.	No.	Mean	D.
Gestational time, weeks									
<33 (mean 30)	7	8 9 (6.4–14 4)	2.9	8	9 1 (4 2 20.3)	5 0	5	12.5 (7.2–16 5)	3.8
33–34 (mean 33.6)	14	6.3 (3 7–12.5)	2.4	6	5 1 (3.3–8.3)	1 9	16	8.8 (5 4–21 8)	4 0
35–40 (mean 37)	12	9 6 (4 0–21 5)	5 1	8	9.5 (4.3–22.5)	6.1	14	15.0 (4 8–36.0)	8.7
Age, weeks	1			2			3		
	No.	Mean	S D.	No.	Mean	D.	No.	Mean	D.
Gestational time, weeks									
<33	7	11 2 (6.1 20.8)	6.6				4	21 7 (8.5–34.6)	12 4
33–34	15	17.9 (11.3–36.0)	7 4	5	37.2 (20.3–60.5)	15.8	10	33.9 (24.9–55.8)	9.5
35–40	10	25 4 (10.0–56.3)	14 6	5	40.9 (28.0–48.3)	8.3	4	44.5 (22.5–80.5)	25 1

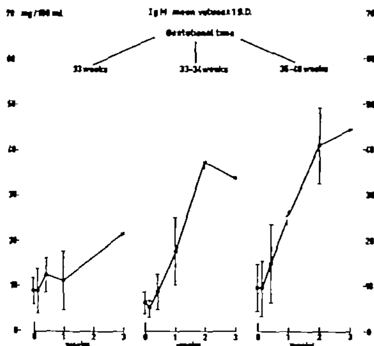


Fig. 4. Average IgM concentrations ± S.D. up to the age of 3 weeks. The infants are grouped according to gestational age.

(Table 4) which is in fairly good agreement with previous findings in full-term babies (14). Neither did the average IgA levels seem to differ noteworthily from those in full-term infants. 13 infants from all three groups showed an average IgA concentration of 6.0 mg/100 ml at the age of 5–6 weeks whereas in a previous study (7) of healthy full-term infants of 6 weeks of age an average IgA level of 6.9 mg/100 ml was found.

IgD. Among all infants with low birth weights IgD was only found in one infant in whom the IgD concentration was 1.8 mg/100 ml at the age of 3 months. This agrees well with previous findings in full term children (7).

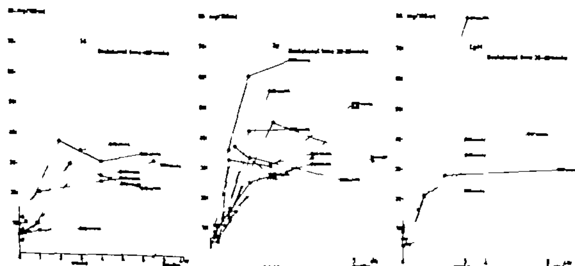


Fig. 5. The development of IgM concentrations in individual infants, grouped according to gestational age. The infants were followed during at least the first 3 weeks of life.

Table 4 *IgA at different ages all infants*

Age, days	0–1	7	14	21	28	35–42
IgA demonstrable	0/35 (0 %)	2/33 (6 %)	4/13 (31 %)	14/18 (78 %)	7/8 (88 %)	13/13 (100 %)
IgA levels, mg/100 ml	—	0–8.4	0–2.9	0–7.5	0–11.1	1.6–13.0

DISCUSSION

In newborn full-term infants the initial IgG concentration is about as high or even somewhat higher than the concentration in the mother (6). The transport of IgG across the placenta is nowadays considered to be an active mechanism which mainly takes place in the course of the later part of pregnancy (6). It would seem of interest, therefore, to study the relation between length of pregnancy and initial IgG concentration in prematurely born infants. In such a study certain sources of error cannot be avoided. A placental transfusion may occur at the time of delivery. The importance of such a transfusion for the initial IgG concentration in the child is difficult to estimate. If it is of any noteworthy magnitude, however, it should result in demonstrable amounts of IgA in the infant and also increased concentration of IgM, as these two immunoglobulins do not normally pass through the placenta to any notable extent. In this investigation, however, only one child was found to have IgA during the first days of life (3.3 mg/100 ml at the age of 3 days). This infant, from whom only one blood specimen was obtained, showed at the same time a strikingly high IgM concentration (51.8 mg/100 ml). Thus a placental transfusion may have occurred in this case. The possibility of an infection in utero cannot either be excluded. The IgA and IgM values of the child are not given in the tables. The child with a gestational age of 31 weeks, however, showed a low initial IgG concentration, 357 mg/100 ml. The gestational age may be difficult to estimate in some individual cases. In one of the infants of the present series it was impossible to calculate the gestational age at all, and the initial IgG concentration in this case is only given in Table 2.

With these reservations, there appears to be a very good correlation between gestational age and initial IgG concentration. This has also been reported by Hobbs & Davis in a preliminary communication (5). The correlation between birth weight and IgG concentration at birth was considerably less pronounced and no statistically significant difference in IgG concentrations between infants with birth weights ranging from 1500–2000 g and those with birth weights of 2001–2500 g was observed. Washburn (15), on comparing prematures of different weight classes found no large difference in the initial IgG levels. In infants with birth weights under 1500 g the level was on the average 41.8 % of the normal adult IgG level, whereas in larger prematures a value of 49.2 % of the adult level was found. As can be seen in Table 1 there were large variations in the IgG levels in all three groups. Isolated high IgG values were also found in infants of low gestational age, while the minimum values rose from group to group. The large variation of the IgG values within the groups support the view that the initial IgG concentration in the infant may also be influenced by other factors than gestational age. It is most probable that the concentration of IgG in the mother at the time of delivery is also of importance in this connection.

From the results of their study Hobbs & Davis (5) arrived at the conclusion that prophylactic gamma globulin treatment is indicated in infants of low gestational age. In children with congenital agammaglobulinaemia increased susceptibility to infection occurs when the level of gamma globulin falls below 100–150 mg/100 ml (10). Children of low gestational age have, on the average, low IgG concentrations at birth, and because of the catabolism of IgG during their first months of life they run a risk of getting a more or less pronounced hypogammaglobulinaemia during the course of this period. There may therefore be reason to give gamma globulin to some infants with low gestational ages but there is no reason in general to administer gamma globulin to infants with low birth weights. There is probably no noticeable positive effect from gamma globulin treatment of immature infants during the very first weeks of life.

It is evident that prematurely born infants

generally have a good capacity for early IgM synthesis, which is apparent from the marked rise in IgM as early as during the first week of life. The fact that IgM levels in full term babies rise rapidly during the first 10 days of life has been demonstrated by Roth (12) who used a semi quantitative method. Washburn (15) found in prematures an average IgM increase from 7.8 mg/100 ml at the age of two days to 17 7 mg/100 ml at the age of one week. These values agree well with the present findings in infants of a gestational age of 33–34 weeks. It is possible that the bacterial population of the gut is responsible for an essential part of the antigenic stimulation during the first days of life, thus giving rise to part of the rapid increase of the IgM values during the first week.

At birth, infants of the lowest gestational age do not have lower IgM levels than other prematures or full term infants. During the first few weeks of life, however the IgM concentrations rise considerably more slowly in these infants. The reasons for this phenomenon may be discussed. A possible explanation is that owing to the special care they are given, with incubators, etc., they are better protected from infections than the larger infants. It is also possible that very immature infants have an inferior capacity for IgM synthesis, which may explain their increased liability to catch severe infectious diseases of different kinds, for instance septic infections caused by *E. coli*.

SUMMARY

The different immunoglobulins G, M, A and D were studied in 65 infants with birth weights less than 2500 g, during their first weeks of life. The initial IgG concentration was very well correlated to gestational age, but by no means so well correlated to birth weight. Infants of low gestational age had, on the average, low IgG concentrations at birth and in the course of their first months of life there was a tendency towards more or less pronounced hypogammaglobulinaemia. Infants with low birth weights had, as a rule, good capacity for early IgM synthesis but the increase in IgM levels during the first few weeks of life was, however considerably less rapid in infants of very low gestational age. This may be due to the fact that such infants are less exposed to infections, owing to incubator care and various kinds of precautions. It may also, however be an expression of an inferior capacity for IgM synthesis in these infants. As regards immunoglobulins A and D in infants with low birth weights, no pronounced differences were found on comparison with previous findings in full-term infants.

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MOTOR CONDUCTION VELOCITIES IN NEWBORN INFANTS OF VARIOUS GESTATIONAL AGES

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Calculation of the gestational age of a newborn from the data of the last menstruation is quite frequently difficult. Therefore, birth weight has been extensively used as a criterium of maturity at birth. However birth weight is also an unreliable index of maturity (15), and this is the reason why there has been an increasing interest in assessing the degree of maturity at birth (as an index of postmenstrual age) by other methods, which have been reviewed by Mitchell & Farr (20).

While searching for simple criteria of maturity at birth, as an index of postmenstrual age, it is natural to turn to the peripheral nervous system, partly because extensive studies, concerning the postnatal development of the peripheral nervous system, and spinal reflex mechanisms in the kitten are now available. See Skoglund (28) for a review of these studies which might also form a basis for evaluation of the postnatal maturation in man. Some studies of the motor conduction velocities and of the spinal reflexes, in human infants and children (1 13 30), have also been performed. These works deal mainly with the characteristics and development of healthy fullterm infants, even if some studies on premature infants have been published (4 23 30)

In this report, we are presenting the results of studies of the motor conduction velocities, in the ulnar and peroneal nerves, in newborn infants of different gestational ages.

MATERIAL AND METHODS

The material studied consists of 45 newborn premature (birth weight 2500 grams or below) and fullterm infants. They were examined between the 1st and the 9th day following birth, mostly around the 3rd or the 4th day. Five of these infants were later excluded, on account of too great uncertainty in determining the latencies on the photographs, from one of the stimulating points. The remaining 40 children are presented in Table 1. They were partly selected among newborn babies from the Department of Obstetrics at the University Hospital in Umeå. In these cases, both pregnancy and delivery were normal as well as the postnatal period. The rest of the material was taken from the Neonatal Ward of the Department of Pediatrics of the Hospital, and includes cases of asphyxia during or after delivery, infants of low birth weight, and infants with hyperbilirubinaemia. However only three of the infants mentioned last had serum bilirubin levels above 15 mg/100 ml at the time of the examination (see Table 1)

The examinations were performed in a laboratory which had a temperature of at least 20°C. A lamp was placed at about 30 cm from the infants in order to avoid cooling. No special procedures were used to obtain the same skin temperature in each individual case. The skin temperature was recorded in three points, at the stimulating and recording electrodes, immediately before and after each investigation (see Discussion). Intramuscular temperature was not recorded. A few premature infants were studied while lying in incubators. Electrical stimulation was performed, at the usual places, for the nerves concerned, i.e., with the cathode immediately proximal to the elbow, wrist, knee and ankle joints respectively, thus always on the right side. The anode was placed on the back of the forearm or of the foot/leg. The cathode was made of felt which had the form of a cone with a small tip, thus allowing an almost pointed stimulation of the nerve. The ground electrode was a pipe cleaner soaked in saline solution and twisted around the wrist or the ankle between the distal stimulating point and the recording electrode. The electrode was a concentric needle electrode (DISA type 9013K0511), inserted in the belly of the hypothenar or the short toe extensor muscles. A needle electrode was chosen because, during the preliminary experiments, it was found difficult to obtain well defined potentials with surface electrodes.

The stimulus was a rectangular pulse, having a duration of 0 (exceptionally 0.5) msec, delivered from

Table 1 *The material investigated.*

The double information in cases 38–40 refers to the two examinations performed on these infants. For further explanation, see the text

Number	Sex	Length of gestation, days	Birth weight, grams	Post-menstrual age in days (at the examination)	Age in days, after delivery	Pre- or postnatal asphyxia, or both	Conduction Velocity Ulnar nerve, (m/sec)	Conduction Velocity Peroneal nerve (m/sec)
1	boy	294	3870	301	7	–	34	29
2	boy	295	3730	300	5	–	35	31
3	boy	299	3610	300	1	+	29	27
4	girl	298	3450	299	1	–	28	22
5	boy	293	4200	298	5	–	30	—
6	girl	279	3780	298	9	–	24	—
7	girl	292	4050	294	2	–	27	22
8	boy	288	4410	292	4	–	30	27
9	boy	284	4020	290	6	+	41	30
10	girl	288	3450	289	1	+	34	25
11	girl	283	3500	288	5	–	29	28
12	girl	285	3390	287	2	–	30	23
13	girl	286	3200	287	1	–	25	25
14	girl	284	3450	286	2	–	29	26
15	boy	278	3260	286	8	+	—	33
16	girl	281	4300	284	3	–	29	25
17	boy	283	3420	284	1	–	26	23
18	girl	279	3220	282	3	+	36	23
19	girl	264	3290	281	7	+	31	26
20	girl	275	3025	279	4	+	33	28
21	girl	277	4050	278	1	–	35	25
22	girl	277	3270	278	1	–	30	27
23	boy	276	3160	278	2	–	23	26
4	girl	271	3730	273	2	–	26	20
	girl	271	3470	272	1	–	29	21
26	girl	272	3330	272	0	+	30	24
27	boy	270	3160	271	1	–	23	18
28	girl	266	1985	269	3	–	21	20
29	girl	258	2320	263	5	–	21	19
30	girl	257	3020	261	4	–	22	18
31	boy	253	2610	255	2	+	21	16
32	girl, twin	249	2040	255	6	+	21	17
33	boy twin	249	1940	255	6	–	22	20
34	girl	244	2380	251	7	–	15	16
35	girl	244	2060	251	7	–	23	19
36	boy twin	246	470	250	4	+	25	19
37	girl, twin	246	2430	250	4	–	19	17
38	girl I	237	2150	244	7	–	21	14
	II			263	26		26	29
39	boy I	237	1540	240	3	+	18	19
	II			268	31		28	21
40	boy I	233	1980	236	3	–	20	19
	II			258	25		26	23

Serum bilirubin levels bove 15 mg/100 ml t the time of the examination were noted in cases 2 (16 9), 11 (18.0) and 36 (16.5).

DISA Multistim, through double-shielded transformer The skin was thoroughly cleaned before the stimulation, in order to minimize the resistance. (The stimulation current was not measured) The voltage chosen was approximately twice maximal to excite the fastest conducting motor fibres, in the nerves under study

The muscle potentials evoked were amplified in DISA 2-Channel Electromyograph (Type 14A20) and were also displayed on Tektronix Oscilloscope, Type 502A. A Polaroid camera was used for photographing. The sweep speed was 2 msec/cm.

The photographs allowed determinations of the latencies between shock artifacts, and the initial deflection of the evoked responses (from which the conduction velocities

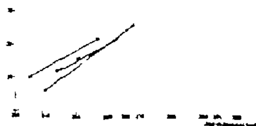


Fig. 1. Motor conduction velocity in the ulnar nerve plotted against the postmenstrual age in days, at the time of the examination. Vertical dotted lines: limits of "normal" gestational age. Filled circles: girls, birth weight over 2500 g. Filled squares: boys, birth weight over 2500 g. Open circles: girls, birth weight of 2500 g or less. Open squares: boys, birth weight of 2500 g or less. The lines between two marks join the results of two examinations conducted on the same child.

were calculated) with an accuracy of 0.2 msec. The distance between the points of stimulation was measured with an accuracy of 1 mm.

RESULTS

The whole material is presented in Table 1, which includes information about sex, birth weight, age in days from the first day of the last menstruation to the day of the examination (in the table called postmenstrual age in days), age in days after delivery, notes on occurrence of asphyxia, and finally the conduction velocities in the ulnar and peroneal nerves. It seems appropriate to briefly comment here upon the findings presented in this table.

A. *Ulnar nerve*

The results are graphically shown in Fig. 1. For the material taken as a whole, there exists a significant correlation between the age in days after the first day of the last menstruation and the conduction velocity ($p < 0.001$). The correlation coefficient is 0.77 and the equation of the regression line of y on x is $y = -34.51 + 0.223\ x$, where y is conduction velocity and x is postmenstrual age at the time of the examination.

1. *Infants of short gestational a*[illegible] *below 266 days).* The group consists of 1[illegible] [illegible]dren. Most infants of short gestational age (in [illegible] material, practically all of them have low birth weights as well) have lower conduction velocities than the fullterm infants, even if there are a few exceptions. Three of these infants have been examined twice. The two different results obtained are joined with a line in Fig. 1. The results of the second examination fall well within the predicted levels for the actual age. The mean value for the whole group of infants of short gestational age (below 266 days) is 20.7 m/sec ($n = 12$, S.D. 2.53).

2. *Fullterm infants.* This group consists of a total of 27 children. Six of them were examined at a postmenstrual age, above 294 days. There is, however, no difference with regard to the variation between the low and the high values of the conduction velocities, among these six infants compared with those examined at a normal postmenstrual age. There are even a few low values among the former ones. The mean value for the whole group of infants of normal or slightly prolonged postmenstrual age, is 29.5 m/sec ($n = 27$, S.D. 4.52). None of these children was examined twice.

B. *Peroneal nerve*

The results obtained for this nerve are graphically presented in Fig. 2. There is also in this case a significant correlation between the postmenstrual age and the conduction velocity ($p < 0.001$). The correlation coefficient is 0.78, and the equation of the regression line of y on x is $y = -33.61 - 0.204\ x$, where y is conduction velocity and x is postmenstrual age at the time of the examination.

1. *Infants of short gestational age.* The group consists of 12 children. Here the results are very much alike those obtained for the ulnar nerve, i.e., most premature infants have low conduction velocities, compared with the fullterm infants. Three of the infants were examined twice. The two different measurements are joined with a line in Fig. [illegible] Two of the three results, obtained at the second examination, fall within predicted values for the actual age. The reason of a marked elevation of conduction velocity at the second examination, in one particular case, is not known. The

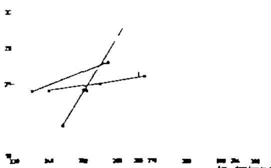


Fig 2 Motor conduction velocity in the peroneal nerve, plotted against the postmenstrual age in days at the time of the examination. For further explanation, see legend of Fig. 1

mean value for the whole group is 17 8 m/sec (n = 12, S.D. 1 76). This mean value is significantly lower than the mean value obtained for the ulnar nerve in the same children ($p < 0\ 01$ in paired comparison)

2. *Fullterm i fants* The whole group comprised 26 infants. Four of the children in this group examined at a postmenstrual age above 294 .. No difference was found between these children and those examined within normal time, as was the case for the ulnar nerve. The mean value of the conduction velocity is 25.2 m/sec (n = 26, S.D. 3.57).

As can be seen from Table 1 we found in most cases lower conduction velocities in the peroneal nerve than in the ulnar nerve in the same child. There is also a significantly lower mean value, $p < 0\ 001$ for paired comparison.

DISCUSSION

Among the factors which might have influenced these results and thus be partly responsible for the variations between the values obtained from various infants, there are two factors which must be commented upon before further discussion. These consist of errors in determination of the conduction velocities and uncertainties in defining the gestational age.

Errors in the measurement of both the latencies and of the distance between the stimulation points, might of course occur These two types of errors have a tendency to be constant under the experimental conditions described. We have calculated these errors to be 0.2 msec and 1 mm respectively which gives a variation in conduction velocity of about ± 2 m/sec. They cannot possibly affect the noted differences to any higher degree.

The age of each infant at the time of the examination was calculated from the date given for the first day of the last menstruation. These figures were obtained from the obstetrical records, and were not verified by personal interviews with the mothers. This method of assessing length of gestation has its well-known limitations. The dates of the last menstruation might be false and, furthermore, the time between ovulation and menstruation varies. A control of the figures, with regard to the beginning of the last menstruation by direct questioning of the mothers, might have justified the exclusion of some cases (on account of a too great uncertainty of the figures obtained) and therefore less varying results.

a. *Fullterm infants.* As far as these babies are concerned, the results obtained in this study differ very little from those described by Thomas & Lambert (30) and Gamstorp (13) In accordance with Gamstorp and in contrast with Thomas & Lambert, we found slightly lower conduction velocities in the peroneal, than in the ulnar nerve.

As it is well known, there exists a correlation (found in several studies performed on animals) between the diameter and the conduction velocity of the nerve fibre (18) This correlation has recently been discussed by Skoglund (27) and by Ekholm (8) who found, at least for the growing cat, a slightly lower correlation factor than the one given by Hursh (18) It would therefore be interesting to compare the calibre spectra of peripheral nerves with the conduction velocities actually also found in man in this report. Some information is available which can be used in this respect. Nyström & Skoglund (21) investigated the fiber diameters of the nerve roots L 2–S 1 and some peripheral nerves, in the lower extremity of five newborn infants, who died in the immediate postnatal period. Unfortunately we could not study the nerves which were analysed by Nyström & Skoglund. It can be justified however to compare the calibre spectra, found by Nyström & Skoglund, of the nerve going to the gastrocnemius muscle, with the motor conduction velocities found

in our study in the peroneal nerve. The peak in the calibre spectrum of the gastrocnemius nerve is situated around 6 microns (even if a few larger fibres are found), which corresponds to a motor conduction velocity slightly above 30 m/sec in this nerve, if the correlation factor of Ekholm (8) is used.

As indicated in our results, there exists a correlation between the motor conduction velocities and the postmenstrual age. There is however a rather great variation in the results found in infants of the same gestational age. This might be to some extent, due to the errors discussed previously An other important factor might also be a real difference in conduction velocity due to individual variation. Such differences are known from the experiments conducted on kittens by Skoglund, in which the experimental situation could be more exactly controlled. Rather great variations were found, both with regards to motor abilities and conduction velocities even among kittens from the same litter (26 27). Available experimental evidence thus points to biological variations as an explanation for at least some of the individual differences noted in our material. The possible influence of other mechanisms must also be discussed. In this context, one must note, however that newborn kitten and newborn man differ with regard to morphological and simple physiological functions of the nervous elements, on one side, and motor abilities, on the other (1 21)

As mentioned earlier skin temperature was recorded in three points, along the extremities under study in each child, before and after the investigation. It was found that the skin temperature varied considerably (up to 2–3 C) between different points, at the same time, and also at the same point before and after the examination. In the same part of one extremity the temperature of the skin is probably not correlated with the actual intramuscular temperature. Thomas & Lambert (30) concluded from their studies of intramuscular temperature: "temperature variations were not an important factor affecting differences of conduction velocity of nerves among the newborn infants The most significant temperature in this context, i.e. the temperature of the nerves, cannot be measured or controlled in man. With these facts in mind, we have chosen not to present the results in detail of our recordings of the skin temperature. It can be mentioned however that in none of the two groups, any correlation was found between high conduction velocity and high skin temperature, for example

The condition of the child during labour and at birth, does not seem to influence the conduction velocities at the time when they are performed. In Table 1 all cases with asphyxia during or after birth are indicated. The results obtained from these children fit very well with those obtained for the whole group A more pronounced asphyxia at birth, with Apgar point 3 or less one minute after delivery was noted in cases 18 and 20 among the fullterm infants (as in cases 31 and 36 among the premature infants). Ten minutes later only one of these patients was seriously asphyctic. These infants also seem to have conduction velocities within expected limits.

During the first few days of life the neurological status is said to have its special characteristics, "birth shock (10) Therefore, the age in days after delivery at the time of examination, is presented in Table 1 In this small material, no infants examined shortly after delivery had unexpectedly low values of the conduction velocities.

The effect of high bilirubin levels is difficult to evaluate, since a hyperbilirubinaemia was only noticed in cases 2 and 11 (case 36 among the prematures). The conduction velocity does not seem to be affected.

From these results, we can conclude, that there exists a variation among fullterm newborn infants, with regard to motor conduction velocity regardless f the errors discussed earlier This variation is partly due to different gestational ages, but within all probabilities, also partly due to variations in the maturation, of other nature, of motor nerve fibres at birth and shortly thereafter Furthermore, the motor conduction velocities in the upper extremity are generally faster than those in the lower extremity This might also be due, in man, to differences in the maturation of nervous elements, between arm and leg, at birth, like the differences found by Skoglund & Romero (29) and Ekholm (8), in the measurements of nerve fibre diameters in kittens.

b *Infants of short gestational age* Conduction velocities in premature infants have earlier been investigated by Thomas & Lambert (30), Cerra & Johnson (4) and Ruppert *et al.* (23). Our mean value for the conduction velocity in the ulnar nerve

is in good agreement with that found by Thomas & Lambert and Cerra & Johnson. It is significantly lower than the mean value for the fullterm infants. Also for the peroneal nerve the mean value for the infants of short age is lower than that of the fullterm infants. In contrast to Cerra & Johnson (4) we found a statistically significant difference between the mean values for the ulnar and peroneal nerves.

Three of the infants were studied twice. All the values obtained, during the second examination of the ulnar nerve fall within the expected values for the age. This would indicate that the maturation process of the peripheral nerves continues, at an unchanged speed after delivery at least until the time of expected birth, cf. Ruppert *et al* (23). The discrepancy between the expected and observed value at the second examination of the peroneal nerve, in one particular case, is difficult to explain. More studies are necessary in order to evaluate the changes in conduction velocity in the neonatal period, as well as its relation to the birth process

The same explanation can be given for the infants of short gestational age, as it was stated earlier for the fullterm infants, concerning the rather large variation obtained between different children.

Conduction velocities as criteria of gestational age

Michell & Farr (20) reviewed the problem of judging fetal age from examinations of the newborn baby Different methods have been used. As it was already mentioned in the introduction the birth weight is an unreliable index of gestational age. A roentgenological examination of epiphyseal centers is probably the most common objective method, the foetus mostly being examined in utero. Unfortunately this method is subjected to important limitations, as the ossification can be delayed in such cases as foetal malnutrition (25) or toxaemia in pregnancy (16). External characteristics as an index of maturity (or gestational age) have especially been investigated by Farr *et al.* (11 12) who found a rather good correlation between the "total maturity score and the gestational age (error less than ± 2.4 weeks in 95% of the cases). This correlation was found to be better than the one found between birth weight and gestational age (*error less than* ± 3.0 *weeks*)

The behaviour of the child, taken from the neurological point of view as an index of maturity was already described by Gesell & Amatruda (14). Saint-Anne-Dargassies (24) has later carefully studied the neurological development of premature infants, and pointed out that an infant, born at 6 months of foetal age, reaches the maturation of a fullterm newborn at the age of 3 months, i.e. at the supposed time of delivery Her results have led to several investigations in which the neurological status has been used for assessing the post natal age of a newborn infant (2, 17 22)

Electroencephalographic recordings have been used in determining the gestational age, for a review see Dreyfus-Brisac (7). A fair correlation between the latency of photically evoked responses recorded over the occipital cortex and the gestational age was found by Engel (9) but it was possible to measure the latency in only 75 per cent of the cases, unless a computer was used.

Brody (3 4) found a correlation between the gestational age and the content of foetal hemoglobin in the blood from the umbilical cord. He found it possible to estimate the duration of the pregnancy with a 95 per cent confidence interval of ± 18.5 days ($y-\bar{y}$) The method described seems rather simple but its usefulness in practical work is limited, if cord blood has to be used. Kirschbaum (19) found an even more narrow range (± 13.9 days), using the elaborate method of column chromatography to measure the foetal hemoglobin.

The results obtained in our study show that the technically relatively simple method of determining motor conduction velocities in peripheral nerves, might be of value in assessing the gestational age of a newborn infant. It is however necessary to perform further studies on a larger material. The presented material contains very few infants having a birth weight quite below the one expected for the actual gestational age, and it is therefore difficult to know if intra-uterine retardation of growth, for example, affects the conduction velocity In newborn animals, it is known that undernutrition affects the myelination in the central nervous system. Brain weight is affected to a smaller extent (6) It is also known that intra uterine growth retardation affects the brain weight of human infants to a much smaller degree than the weight of other organs (15). Nothing has yet been found, in man, about the possible influence of an intra-uterine growth retardation upon the

peripheral nervous system. The 95% confidence limits, for assessing the gestational age at mean values of motor conduction velocity are ± 37.1 (ulnar nerve) and ± 28.8 (peroneal nerve) days. In this small material, there is an astonishingly good correlation between the birth weight and the gestational age, the correlation coefficient being 0.86 and the 95% per cent confidence limits for assessing gestational age at mean values of birth weight is ± 23.3 days. This is narrower than that found by other authors (3 4 11) and will probably not be found in a larger material.

A new and larger group of infants is presently under study. We have now limited the study to the ulnar nerve, in which, conduction velocity excitability and refractory time, are measured. We also intend to study the influence of gestational age, birth weight, nutritional status, sodium, potassium and calcium levels. At the same time and on the very same children one of us (O F) is studying other criteria for maturity such as external characteristics, neurological status mainly based upon the criteria given by Saint Anne Dargassies (24), roentgenological examination of epiphyseal centers, and foetal hemoglobin levels in cord blood. We consider that it would be interesting to compare different methods of assessing maturity in the same children, in order to obtain a better understanding of the influence of the gestational age, the nutrition and other factors, on the criteria mentioned.

SUMMARY

The motor conduction velocities, in the ulnar and peroneal nerves of newborn infants of various postmenstrual ages were studied. It was found that.

1. The conduction velocities, in children of a short gestational age is significantly lower than those of fullterm infants.

2. The conduction velocities, of the peroneal nerve, is slightly but significantly lower than those of the ulnar nerve in both groups of children.

It is also concluded that the determination of motor conduction velocities seems to be an additional method, of value, in assessing the degree of maturity at birth, and in determining the gestational age.

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THE ANTIBODY PATTERN IN REPRESENTATIVE GROUPS OF ETHIOPIAN VILLAGE CHILDREN

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Since 1962 the Children's Nutrition Unit (CNU)[1] financed by the Swedish International Development Authority (SIDA) in collaboration with the Imperial Ethiopian Government, has been carrying out investigations in Ethiopia (1). These have been especially directed towards the incidence and type of undernutrition in children from birth up to and including the age of ten years, and towards the food which they consume, all with the aim of developing a program of dietary improvement based on ingredients produced within the country. The investigations have also included, obviously, an analysis of the general state of health of the children. As a part of these investigations it was considered essential to determine the incidence of antibodies against a number of relevant pathogens in order to obtain an idea of the previous disease history and present state of immunity in these children.

For the various activities of the project CNU has started field centres and field stations in different parts of the country. In the two field centres, medical examinations have been carried out weekly and the children have been followed up with longitudinal studies, while at the field stations the investigations have been of a cross-sectional nature. Of the field centres, one lay in Addis Ababa relatively near to the CNU base laboratory and the other still lies in the village of Ijaji 215 km west of the capital. The three field stations are located in the provinces of Tigré, Arussi and Sidamo (see map, Fig. 1). Each of the field centres and field stations represents different cultures and living condition. The area belonging to the field centre in Addis Ababa can be regarded as typical of the Ethiopian city environment, and Ijaji is representative of the medium-sized market village with a neighbouring agricultural population on the central Ethiopian highlands. The main crops cultivated here are corn, sorghum and *teff (Eragrostis abyssinica)*. In Tigré a small village has been chosen on the dry and completely treeless high plateau. The inhabitants live under primitive conditions in small stone houses, they grow rye, wheat and *teff* and have a small number of livestock. In the province of Arussi a region close to the Zwai lake has been chosen, where the inhabitants of the savannah, which is very dry during the greater part of the year, are half nomadic and able to cultivate very little, but have large herds of cattle. In the third station, in Sidamo, the environment is quite different. The people here live on *ensete*, a substance prepared from the false banana (*Ensete ventricosum*), and containing mainly carbohydrate with only one per cent of protein. This plant is cultivated in a verdant landscape, where the densely located houses are each surrounded by an ensete orchard, and where the cultivation of coffee comprises a considerable source of extra income.

The different regions lie at altitudes of between 1700 and 2350 metres. The day temperature is, at its highest, between 25 C and 35 C, while the night temperature is about 12–14 C. This applies to the greater part of the year. During the rainy period, which occurs at slightly different times in the different localities in question, the temperatures, especially at night, are considerably lower.

The diseases for which antibody studies were considered of value were poliomyelitis, morbilli, rickettsial diseases, pertussis, streptococcal infection, salmonellosis, syphilis, toxoplasmosis, and schistosomiasis.

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Serological Tests

The analyses are performed partly at the Institute of Medical Microbiology Upsala, and partly at the State Bacteriological Laboratory Stockholm.

1. *Poliomyelitis*

Neutralization was carried out by immuno inactivation of five fold dilution of the sera and with the phase-system technique as described by Philipson et al. (36). Immuno inactivation was used for qualitative demonstration of antibodies, and the phase system technique was used to estimate the titres in 43 cases.

2. *Morbilli*

Measles antibodies were determined by the hemagglutination-inhibition reaction as modified by Norrby (31).

3 *Rickettsial diseases*

Complement fixation was carried out according to the micromethod of Sever (38). Q fever (*R. burneti*), rickettsial pox (*R. akari*), and epidemic typhus (*R. prowazeki*) antigens, and also control antigens, were kindly supplied by Lederle Laboratories, Pearl River New York.

4. *Pertussis*

An agglutination test was used for antibody titration. The tubes were incubated at +37°C for 1 hour, and the values read after further storage for 18 hours at +4°C.

5 *Streptococcal infection*

Antistreptolysin titres (AST) were determined chiefly as described by Ipsen (20). The results, however are presented as proposed by Packalén & Bergqvist (33) for antistaphylolysin (ASta) determinations.

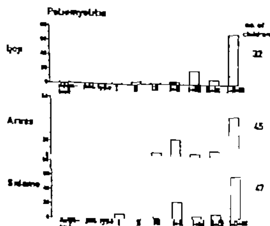


Fig 3 Antibodies to poliomyelitis virus. Percentage distribution of children in Ijaji, Arussi, and Sidamo, in relation to different types and combinations of types of virus

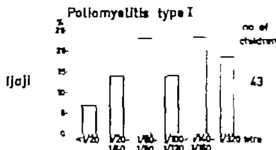


Fig. 4 Antibodies to poliomyelitis virus, type I Per centage distribution according to antibody titre of 43 children in Ijaji, 3–11 years of age.

6. *Salmonellosis*

An agglutination test was performed according to the standard method, and only the somatic antigens representative of the B, C and D groups of Salmonella bacteria (22) were used.

7 *Syphilis*

Kline test was performed according to the standard technique.

8. *Toxoplasmosis*

T xoplasma antibodies were determined by the dye test as described by Sabin and Feldman (37) with some modifications (18).

9 *Schistosomiasis*

Complement-fixation was carried out according to the micromethod of Sever (38). The antigen made from lyophilized adult *S mansoni* worms was prepared as described by Falkenvedan et al (11).

RESULTS

No sex differences were noted in the different investigations, and the results are therefore pre sented w thout sex classification

1 *Poliomy litis*

The incidence of antibodies to poliomyelitis virus is given in Fig. 3 As can be seen in this figure, the incidence of antibodies to all three types was very h gh this value being 69% for the children in Ijaji The correspond g values for Arussi and Sidamo were 56% and 60% respectively No children between 5 and 11 years of ge were completely w thout poliomyel tis antibodies, but among a small group of 11 children from Ijaji 3–4 years old, one child was completely negative. The antibody titres for poliomyelitis irus type I

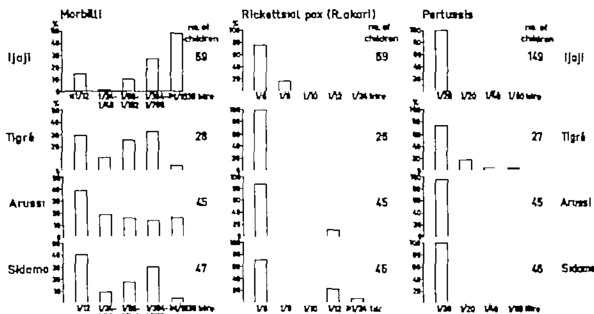


Fig. 5. Antibodies to morbilli virus, to *Rickettsia akari* (rickettsial pox), and to *Haemophilus pertussis*. Percentage distribution of children in the different regions according to titre level.

Table 2. *Antibodies to Rickettsia akari R. burneti and R. prowazeki*

Percentage distribution of children in the different regions according to antibody titre

	Ijaji	Tigré	Arussi	Sidamo
No. of children	59	26	45	45
Rickettsia akari				
Titre				
<1/6	76	100	89	71
1/8	17	0	0	0
1/10	3	0	0	0
1/12	3	0	11	22
≥1/24	0	0	0	7
Rickettsia burneti				
Titre				
<1/6	86	100	91	96
1/8	5	0	0	0
1/10	3	0	0	0
1/12	5	0	7	2
≥1/24	0	0	2	2
Rickettsia prowazeki				
Titre				
<1/6	86	100	91	98
1/8	7	0	0	0
1/10	3	0	0	0
1/12	3	0	9	0
≥1/24	0	0	0	2

were determined on 43 sera from Ijaji (Fig. 4), this group also including the 11 younger children. Three children were found to be negative, with a titre of <1/20 but otherwise the titres were high, in 42% they were ≥1/140.

2. *Morbilli*

The incidence of antibodies to measles virus is given in Fig. 5. In Ijaji 14% of the children were negative, with a titre of <1/12, while 48% had a titre of ≥1/1536 and 27% ≥1/3072. In the other regions the frequency of positive children was lower, being lowest in Sidamo where 60% were positive. But in these regions also, high antibody titres were noted.

3. *Rickettsial diseases*

The antibodies to *Rickettsia akari*, *Rickettsia burneti* and *Rickettsia prowazeki* were determined in 175 sera from the four different regions. Fig. 5 shows the incidence of antibodies to *R. akari* and Table 2 also includes the values for *R. burneti* and *R. prowazeki*. As can be seen in the figure, very few children were positive as regards *R. akari*—none in Tigré, and between 11% and 29% in the other regions. A similar frequency distribution was found for the two other rickettsial diseases.

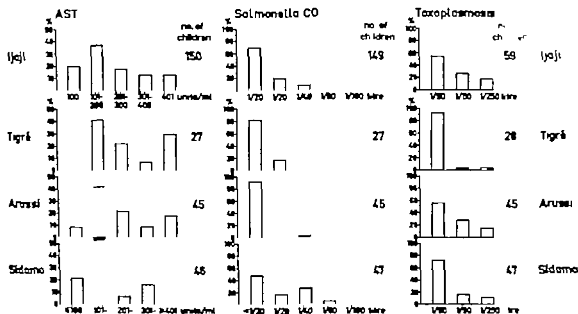


Fig. 6. Antistreptolysin (AST) titres, O-antibodies to the Salmonella C group, and antibodies to *Toxoplasma gondii*. Percentage distribution of children in the different regions according to titre level.

4 *Pertussis*

As seen in Fig. 5 practically all children were negative as regards pertussis antibodies. Of the total 267 children 258 showed no antibodies. In Ijaji and Sidamo none of the children were positive, while 26 % of the small series from Tigré and 4 % of the Arussi series exhibited raised or equivocally raised titre values.

5 *Streptococcal infection*

The results of the antistreptolysin (AST) determinations are shown in Fig. 6. The incidence of raised titres was high in all four regions. In Ijaji, 43 % of the children showed values of over 200 units/ml, while the corresponding figure for Tigré was 59 % for Arussi 49 % and for Sidamo only 4 %. The incidences of titres exceeding 400 units/ml in the different series were 13 % 30 % 18 % and 0 % respectively.

6. *Salmonellosis*

Fig. 6 gives the distribution of Salmonella CO titres in the different regions, and Table 3 also includes the BO and DO titres. With regard to positive CO titres, this incidence was highest in Sidamo, where 51 % showed values of 1 20 or higher and lowest in Arussi, where the corresponding figure was 7 %. The incidence of positive Salmonella BO and DO titres was distributed similarly with the lowest percentage of positive children in Tigré.

7 *Syphilis*

The Kline test was performed on sera from 313 children, of whom only one was found to be positive, an eight-year old boy in Ijaji.

8 *Toxoplasmosis*

The results are given in Fig. 6. The lowest incidence of positive children was found in Tigré, where antibodies were found in only 7 % in the other regions 28–46 % were positive. With the exception of Tigré, the distribution of positive titres was similar in all areas.

9 *Schistosomiasis*

The incidence of antibodies to *Schistosoma mansoni* was studied in 213 sera, all of which were found to be negative.

DISCUSSION

1 *Poliomyelitis*

Until 1960 the public health authorities and physicians Ethiopia considered that poliomye-

Table 3 *O-antibodies of the Salmonella B C, and D groups*

Percentage distribution of children in the different regions according to antibody titre

Region	Ijaji	Tigré	Arussi	Sidamo
No. of children	149	27	45	47
Salmonella BO				
Titre				
<1/20	75	89	84	85
1/20	11	4	4	6
1/40	13	4	11	6
1/80	1	4	0	2
1/160	0	0	0	0
Salmonella CO				
Titre				
<1/20	70	82	93	49
1/20	20	19	2	17
1/40	9	0	2	28
1/80	1	0	2	6
1/160	1	0	0	0
Salmonella DO				
Titre				
<1/20	71	85	64	72
1/20	17	0	22	15
1/40	9	15	7	13
1/80	2	0	7	0
1/160	1	0	0	0

litis did not occur in their country The reason for this belief was obviously the inadequate development of public health services, but also the ... that a large number of children become infected while they are still completely or partly under the protection of diaplacentally transferred antibodies, and that the not so numerous clinically manifested cases are easily mistaken for other febrile diseases. In a series of 126 children with post-poliomyelitic symptoms collected at the Ethio-Swedish Pediatric Clinic (3) the disease in 59% had become manifest between the third and eighteenth months of life. Serological investigations in Addis Ababa (2) revealed the picture characteristic of tropical and subtropical countries, which had been demonstrated previously in Morocco and Egypt, *i ter alia* (34 35) Just over 80% of the Ethiopian children had passively transferred antibodies at birth, but at the age of 6 months such antibodies were only found in approximately 30% Subsequently antibodies re appeared rapidly through natural infection. At the age of one year 93% of the children had antibodies to type III, and before the age of 3 years a very high percentage were also positive for types I and II. In our investigations the incidence of poliomyelitis antibodies was determined in series from Ijaji, Arussi and Sidamo (Fig. 3). The results show similar conditions to those found in the Addis Ababa study despite the different living conditions and the different degree of isolation. The children in Arussi, especially live under conditions greatly different from the densely populated surroundings in the capital. None of the 124 children of the present series completely lacked antibodies to poliomyelitis, and 56% to 69% were positive for all three types. The antibody titres of the Ijaji series were found to be high, which is further evidence of the wide distribution of poliomyelitis virus in the Ethiopian community

2. *Morbilli*

One of the more serious diseases in childhood in the developing countries is measles. In a comprehensive study from West Africa (30) it is stated that half of the children developed measles before the age of a year and eight months. The frequency of complications is considerably higher than that which is usual nowadays in Western Europe and the USA bronchopneumonia occurs in a high percentage, and similarly laryngitis and diarrhoea with dehydration. Further the disease seriously affects the nutritional status of the child and often provokes a latent kwashiorkor (28). The overall mortality in the West African series was 1.3% with considerably higher figures during the first years of life. In a study from Guatemala (14) the mortality was 11.5% among the measles cases during the first year of life, and 8.5% during the second year The mortality figures for hospital series are even higher As an example it may be mentioned that Hendrickse and Sherman (16) report a mortality of 46.6% from University College Hospital, Ibadan, with almost twice as many girls as boys among the deaths. *Clinical experience from Ethiopia has given the* impression that measles certainly occurs, but that it is not such a great problem as in other countries of the same latitude. The serological studies showed that antibodies were present in all regions investigated. The highest frequency was found in Ijaji, where 86% of the children were positive, and the lowest in Sidamo, where the corresponding figure was 60% For comparison it may be mentioned that HI antibodies can be detected in 90%

of children of 5 years of age in an urban population of a European country and at 10 years of age very few susceptibles remain (3). As can be seen in Fig. 5 the Ethiopian titres were high, and this was most pronounced in Ijaji, where 75% of the children showed a titre of ≥1/384. The higher titres in Ijaji were probably connected with the fact that this village has the most active contact with the outer world, compared with the other regions, so that there has been a more continuous influx of measles virus. The reason for the discrepancy between the clinical experience from Ethiopia and the conditions in other tropical countries may be connected with the fact that the degree and type of undernutrition in the ages in question differ in the geographically and culturally widely varying regions.

3 *Rickettsial diseases*

It is generally considered in Ethiopia, both among physicians and the lay population, that spotted fever is common disease, and there are thousands of cases reported annually (10). From time to time small local epidemics occur (42). At the same time it is well known that the customary typhus vaccination does not give the protection that might be expected. The incidence of antibodie against three types of rickettsia was studied, *Rickettsia prowazeki* which causes the common spotted fever *Rickettsia burneti*, which gives Q fever and *Rickettsia akari*, which causes Rickettsial pox. Very few positive titres wer obtained for any of these three types. Fig. 5 shows the incidence of antibodies to *R. akari*. In Tigré 100% were negative, and in the other regions also the incidence of positive titres was very low. It was only in Sidamo that a few per cent showed definitely raised titres, of ≥1/24. The results from the studies of *R. prowazeki* and *R. burneti* were, on the whole, similar. Whether the clinical findings are due to the occurrence of other rickettsial diseases, or whether there is a question of diseases of completely different origin, and in that case mainly anthropod-borne viral fevers, can only be determined by future investigations.

4 *Pertussis*

As in the case of morbilli, whooping cough is of extreme importance in Africa and other tropical countries, with an onset at an early age and a high mortality I a carefully followed up village study in Nigeria (29) 13 children died out of 206 in whom the disease was manifested before the age of five years. The mortality whooping cough at a Nigerian hospital in 19 –1961 was 15.5% in spite of the use of antibiotic Similar figures have been reported from East Africa and India (40), among other places, and from the West Indian island of Antigua (41). Reports from Ethiopia state that in 1958 whooping cough took twelfth place among the diseases diagnosed at some large outpatient clinics in North-western Ethiopia, 0.9% of the patients having this disease (10). We have no definite clinical figures from our own studies, but the disease has been suspected from time to time. A clinical diagnosis is difficult to make in a series where the frequency of bronchopneumonias of varying origin is very high, and when there are no possibilities for bacteriological culture. The results of the antibody investigations are surprising (Fig. 5). In Ijaji and Sidamo all 195 sera were negative, and in Tigré and Arussi there were only few isolated cases with positive sera. The Ethiopian children thus appear to show a strikingly low incidence of pertussis, and an extremely poor stat of immunity at any rate as judged from the serological aspect. Morley *et al* (29) determined the median age in years of notified cases of whooping cough in 17 tropical countries, but not Ethiopia, however and found that this lay between 1.4 and 3.0 years. The present Ethiopian series comprised children of ages 5–11 years. The possibility cannot be precluded, therefore, that several children had had the disease at an early age, but that the agglutinating antibodies, which are of relatively short duration (4), had subsequently disappeared, while clinical immunity had persisted.

5 *Streptococcal infection*

Acute or chronic tonsillitis was seldom observed in the clinical examinations, and no case of scarlet fever was recorded. Neither should this be expected scarlet fever with the disease pattern typical for our countries is rare in tropical climates, while on the other hand inapparent infections are as common or more common in the tropics than in temperate zones. Skin infections of different kinds are very common, on the other hand, due to the poor hygienic conditions and to the very high incidence of mites and other parasites, which result in secondary infection of skin complaints.

The AST titres were determined in 268 children, and as seen in Fig. 6 the frequency of raised titres was high. In the total series 43% of the children showed values of over 200 units/ml, and 13% over 400 units/ml. Approximately similar distributions were found in the different regions, with the exception of Sidamo where the titre level was considerably lower. This could have been due to the more favourable climatic conditions in this region.

6. *Salmonellosis*

In two of the areas, Ijaji and Sidamo, the incidence of positive Salmonella CO titres was comparatively high, 31% and 51% respectively but only in Sidamo titres above 1/20 were found in a moderate number. It should be remembered, however that these agglutination titres do not persist for more than six to twelve months after the end of an infection (27). Fig. 6 shows the incidence of O titres for the Salmonella C group. Between 51% and 7% of the children were positive, with a titre of more than 1/20. The conditions were approximately the same for the Salmonella B and Salmonella D groups. Tigré and Arussi showed the lowest percentage of positive children, which might conceivably be connected with the fact both of these regions are very dry during greater part of the year with consequent ...tion of the spread of bacteria.

In view of the particularly common and dangerous diarrhoeal diseases among Ethiopian children, bacteriological faecal studies were also carried out in Ijaji (17). The results showed that only 2 out of 310 faecal cultures were positive for Salmonella.

7. *Syphilis*

In the available medical statistics from Ethiopia, syphilis, as in many other African countries, takes an important place among the most commonly occurring diseases. Thus in Gondar (21) for example, it has been found that 46.6% of 987 unselected mothers attending the MCH clinic were serologically positive (Kahn and VDRL) and the frequency among 1500 young schoolchildren was 8.2%. In a WHO investigation from 1949 it was found that 18.7% of children of ages 0 to 12 years were Kahn-positive (15) and in Malchew in the northern part of Ethiopia the frequency of VDRL-positive children between 5 and 14 years has been reported as 14.2% (6). The disease pattern in Ethiopia differs, however from the usual course in Europe. The early stages are of the same type, but late neurolues is reported to be completely absent, and cardiovascular lues to be extremely rare. Further it is considered that congenital syphilis never occurs or is extremely uncommon. The latter opinion has been verified by our investigations: we have not seen one clinical case of unequivocal congenital syphilis, in spite of the very large number of infants studied. It has happened on repeated occasions that new mothers have sought advice because they believed that they had syphilis and wished to have their newborn child examined and treated, but the results have always been negative. The Kline test was performed on a material of sera from 313 children from CNU field stations and field centres, and this resulted in one positive reaction, a sample from an 8-year-old boy in Ijaji. This difference in result with earlier investigations is striking and will be subjected to further studies.

8. *Toxoplasmosis*

It was considered of interest to study also the incidence of antibodies to *Toxoplasma gondii* (Fig. 6). The percentage of positive cases varied in the different regions, being about 45% in Ijaji and Arussi, and only 7% in the very small series from Tigré. The incidence of toxoplasma antibodies in different populations varies according to the geographical and climatological conditions, *inter alia*. Thus it is well known that the incidence is extremely low in Arctic regions and increases towards the equator especially in hot humid areas (12, 26). In European studies on corresponding age groups the frequency of positive cases has been found to be 7–14% (19, 26, 39), while tropical populations of the same ages can reach figures of 70–80%. With regard to African conditions, dye test studies from Liberia and East Africa (13), from Nigeria (25) and from Sudan (8) have shown high frequencies of toxoplasma antibodies. These series, however include no or very few sera from children in the age group in question, and no comparisons can therefore be made.

9. *Schistosomiasis*

Both *Schistosoma haematobium* and *Schistosoma mansoni* occur endemically in different regions of Ethiopia, especially around the large Lake Tana

in the north-west (9), in the province of Tigré, not too far from our field station (7), and in a region near Harar in the eastern part of the country (23). In studies at these localities, a very high parasite frequency has been found already at school age. Kubasta (23) reported that 48.3% of a group of boys of ages 9–11 years were infected with *S. mansoni*, and Chang (9) found a frequency of 22.8% for the same parasite among school children in the village of Gorgora at the northern shore of Lake Tana. In the present series, where 213 sera were analysed for *S. mansoni*, all were found to be negative, confirming the results of the clinical investigations in which a very large number of urine sediment and faecal specimens showed no schistosoma eggs. With all probability this disease will become a great medical problem in the future; the snails which serve as intermediate hosts have been found, in surveys, to occur over the greater part of the country up to an altitude of about 2700 metres (5, 24). With the development of planned artificial irrigation programs and increasing mobility among the population, with higher concentration to densely populated areas, it is considered that this disease could rapidly reach the same wide distribution and become as large a medical problem as in the neighbouring countries of Sudan, Egypt and parts of the Arabian peninsula.

SUMMARY

As a part of the comprehensive program of investigation which is being conducted by the Children's Nutrition Unit in Ethiopia, it was considered essential to determine the incidence of antibodies to a number of important pathogens, in order to obtain an idea of the previous disease history and present state of immunity of the children. The material comprised sera from 525 children of ages 5–11 years from those localities in which the project has established field activities.

Poliomyelitis: The incidence of antibodies to poliomyelitis virus was very high, and 56–69% of the children studied were positive for all three types. The titre levels were studied in a small series, and these showed high values.

Morbilli. Antibodies to morbilli virus were found to occur in 60 to 86% of the children in the different regions.

Rickettsial diseases: The incidence of antibodies to *R. akari*, *R. burneti* and *R. p[illegible]* was studied, and in all regions a very l[illegible] frequency of positive titres was found.

Pertussis: The antibody incidence [illegible] very low throughout, and in two regions no antibodies were found.

Streptococcal infection. The AST titres lay at a high level, with 43% of the children over 200 units/ml and 13% over 400 units/ml.

Salmonellosis: The titre levels of Salmonella BO, CO and DO were low and the frequency of positive titres varied between 7% and 51[illegible]

Syphilis: Kline's test was performed on sera from 313 children, and only one positive reaction was found.

Toxoplasmosis. The incidence of positive findings varied between 7% and 46% in the different regions.

Schistosomiasis. The incidence of antibodies to *Schistosoma mansoni* was studied in 213 sera, all of which were negative.

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AN OUTBREAK AMONG CHILDREN OF ASEPTIC MENINGITIS CAUSED BY ECHOVIRUS TYPE 9

A Clinical and Serological Study

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There are several viral diseases, prevalent during summer and autumn among children and adults in temperate zones, which are characterized by high temperature, rash, gastrointestinal disturbances, and signs of meningeal irritation. Increasing evidence, based on improvement in laboratory technique, points to Echovirus 9 as one of the enteroviruses responsible for these diseases.

The purpose of the present communication is to describe a small outbreak, caused by this virus, in which the sole clinical syndrome observed was that of meningitis. The outbreak occurred in the early summer of 1967 in Jerusalem, Israel, affecting a group of children living in a fairly limited area of a residential suburb.

CASE REPORTS

Case 1

P Z., 12 1/2-year-old male, was reportedly in good health until two days prior to admission to the hospital, when he developed irritability, headache and severe vomiting. No improvement was achieved with antibiotic and antipyretic drugs. Two weeks prior to the outbreak of these symptoms he had suffered from chickenpox and pneumonia, from which he had completely recovered.

Physical examination on admission revealed a pale sick-looking child. Temperature was 37°C, pulse rate, 134 per minute; respiratory rate, 30 per minute; blood pressure, 100/65 mm Hg. There was some neck rigidity and Kernig and Brudzinski signs were negative. Tendon and cutaneous reflexes and plantar responses were normal. The tongue was wet and white. Systolic murmur grade 1 was found in the heart. There was no other abnormal findings in the physical examination. T days later his condition became worse, fever remained high, headache was severe, and the child complained of vertigo and dizziness. Physical examination revealed marked neck rigidity and Kernig and Brudzinski signs were positive. Tendon and cutaneous reflexes were slightly diminished, and Babinski's sign was positive on both sides.

Laboratory data. The urine was normal chemically and microscopically. The blood count showed: Hb, 12.7 g/100 ml, hematocrit—40%, W.B.C., 11,500 per mm³ (72% neutrophils, of which 13% were bands, 25% lymphocytes, 4% monocytes). R.B.C. morphology was normal. E.S.R., 38/90. Serum glucose and electrolytes were normal. Findings in the cerebrospinal fluid (CSF) were as follows. Cells—2350 leucocytes (90% lymphocytes) and 5 RBC, per cubic mm, sugar 35 mg/100 ml; protein, 58 mg/100 ml; chloride, 113 mEq/L. Bacterial culture was negative. Viral culture of the CSF yielded echovirus 9.

Evolution. The severe clinical signs which resembled those of septic meningitis, were the reason for the initial treatment which included injections of penicillin, streptomycin, and antipyretic drugs. The antibiotic injections were stopped when it became evident that the disease was the aseptic type, and only symptomatic therapy was continued. 4–5 days after admission the patient condition improved considerably. The temperature subsided to normal limits, signs of meningeal irritation disappeared and the CSF obtained at that time showed normal findings (see Table 2).

Case 2

F L, 10-year-old female, was in good health until 4 days prior to her admission to hospital, when she developed fever accompanied by intractable headache and severe vomiting, and later became somnolent. No improvement in her condition was observed following treatment with antibiotic and antipyretic drugs.

The girl' past history was noncontributory. Her brother (F Z.) had been hospitalized several days before her admission, due to the same symptoms.

Physical examination on admission revealed a somnolent girl, who could not sit up and hardly moved at all. Temperature was 38.5°C, pulse rate, 100 per minute; blood pressure, 110/75 mm Hg. The skin was pale. No

ble 1 *Clinical findings at admission and duration of disease in six children with aseptic meningitis*

Patient se age	Day from onset and date of admission	Headache or irritability	Vomiting	Somnolence	Neck rigidity	Brudzinski's sign	Kernig' sign	Fever	Rash	Electroencephalogram	Total duration of disease (days)
1 F Z., male, 5 years	4 13.3 67	+	+	O	±	±	±	++	Ø	Normal	7
2. F L., female, 10 years	5 20.3 67	++	++	++	++	+	+	++	Ø	Normal	9
3 M Y., male 5 years	3 30.3.67	++	+−	±	++	+	+	++	Ø	Normal	7
4 L. G male, 3 years	4 6.4.67	++	+	O	++	+	+	++	Ø	Not performed	8
5. C. A., male, 15 mo	5 13.4.67	++	Ø	±	+	±	±	++	+	Normal	9
6. B. M., male, 16 mo.	7 15 4.67	±	Ø	O	Ø	Ø	O	+	Ø	Normal	8

rash could be seen. The tongue was coated. Neck rigidity was evident. Kernig and Brudzinski' signs were positive. Tendon and cutaneous reflexes as well as plantar responses were normal. Further physical examination revealed no abnormal findings.

Laboratory data. Urine was negative. Blood count showed: Hb, 13 g 100 ml, RBC, 4,500,000 per mm³ W.B.C., 10,000 per mm³ (66% nucleated polymorphonuclears, 5% bands, 25% lymphocytes, 4% monocytes), ESR., 40 70 Stool culture and throat swab culture negative The CSF obtained at that time was hyper[illegible], cloudy in appearance, and contained 2600 leucocytes (more than 90% lymphocytes) and 4 RBC per mm³ Other values were Sugar 50 mg 100 ml, protein 34 mg/100 ml, chloride 116 mEq/L Bacterial culture of the fluid was negative. Viral culture yielded—echovirus 9.

E olution. In this case too, the severe clinical picture that at first sight was compatible with the diagnosis of septic meningitis (even more than in Case 1) was the reason for the initial treatment of penicillin and streptomycin injections and the administration of antipyretic drugs. The fever subsided to normal limits within 3 days, and the patient general condition improved remarkably

The lumbar puncture performed on the 11th day of hospitalization revealed normal findings in the CSF and the patient was sent home few days later.

Following the hospitalization of the first two patients

Table 2. *Laboratory findings, according to day of illness and to clinical signs in 6 children with the aseptic meningitis syndrome*

CSF – Cerebrospinal fluid, TS – throat sw b RS – rectal swab

Patient	Day from onset	Clinical signs of meningitis	Findings in cerebrospinal fluid: Increased L. P pres	Cells* per mm³	Protein (mg/100 ml)	Sugar (mg/100 ml)	Chloride (mEq/l)	Bacter culture	Virus culture (CSF TS or RS)	Blood W.B.C. (per mm³)
1 F Z.	6	−+	+	2360	58	35	113	Negative	+(CSF)	15,000
	11	None	—	54	26	45		Negative		
2 F L.	6	++	−+	2600	34	50	116	Negative	+(CSF)	13,700
	15	None	—	42	19	40	113	Negative		
3 M. Y	3	++	±	1650	27	62	118	Negative	+(CSF)	10,800
	8	None	—	27	30	50	114	Negative		
4. L. G.	5	++	—	216	14	75	111	Negative	+(CSF)	6,900
5 C. A.	6	++	−	1200	44	42	121	Negative	+(TS), (CSF)	10,100
6. B. M.	7	None	±	500	48	61	110	Negative	+(TS), (RS)	9 000

* More than 90 % of the cells were lymphocytes.

presented above, four more children, aged from 15 months to 5 years, were admitted to our department with similar complaints. 10 days elapsed between admissions, and all the patients were admitted during period of less than 5 weeks (from March 13 1967 to April 15 1967). Clinical and laboratory findings in these additional cases were almost the same as those described in the first cases, but ith varying degree of severity The youngest two children had milder symptoms, and convulsions ere not observed in any of the patients (Tables 1 and 2).

VIROLOGICAL STUDIES

Virus isolation Throat and rectal swabs (TS, RS) taken from the patients, were eluted into phosphate buffered saline, pH 7 4 containing 0.5 % bovine plasma albumin, 1000 units penicillin, 1000 µg streptomycin, and 10 µg fugizone (PBA). The eluates were then inoculated, in 0.. ml amounts, into tubes of primary rhesus monkey kidney (MK) cell culture. Cerebrospinal fluid (CSF) samples were likewise inoculated, but without prior treatment. After an adsorption period of 1–2 hours, at 36°C, medium M199 was added and the cultures were reincubated.

When a cytopathic effect involving most of the cells was evident a passage was made, and stock virus was prepared from it for the purpose of identifying the isolate.

Virus identification. Stock virus from the first two isolates (F Z. and F I.) was inoculated into one-day-old suckling mice. Within week the animals developed clear signs of flaccid paralysis. Since the original CSF specimens had been negative in suckling mice, the presence of ECHO virus type 9 was suspected (9) Neutralization experiments were carried out, using hyperimmune rabbit serum prepared against the echovirus 9 prototype strain (Hill).

0.3 ml of the isolate containing 100 $TCID_{50}$ per 0 1 ml, was incubated for 1 hour at 36 C, with an equal volume f a 1 20 dilution of anti-Hill serum, and 0.. ml aliquotes of the mixture were inoculated into MK cultures No cytopathic effect was observed in these cultures, whereas complete destruction of the cell heet occurred in control cultures, inoculated with the virus alone. The presence of echovirus 9 in the specimen received was thus confirmed.

Serology Sera separated from the blood samples received were stored at − 20 C Before testing for neutralizing antibody content, they were thawed and inactivated at 56 C for 30 minutes.

Table 3 *Echovirus 9 and neutralizing ibody titer in 6 children with clinical manifestatio f meningitis*

Patient	Specimen received	Day from onset	Echovirus 9 isolation	Neutralizing antibody titer
1 F S.	C. S. F	8	+	
	Blood	40	N A.	1 640
2. F I	C. S. F	6	+	
	Blood	28	N A.	1 640
3. M. Y	C. S. F	3	−	
4 L. O.	C. S. F	5	+	
	Blood	19	N A.	1 640
5 C. A.	T S.	2	+	
	T S.	5	+	
	C. S. F	5	+	
	Blood	10	N. A.	1 640
6 B M.	T S	2	−	
	C. S. F	8	−	
	R. S.	10	−	
	Blood I	10	N A.	1 160
	Blood II	40	N A.	1 640

Cerebrospinal fluid
[b] Not attempted
Throat swab
[d] Rectal swab.

Serial fourfold dilutions prepared in PBA were then incubated with virus and the mixtures were inoculated into MK cultures, as described above. The highest serum dilution preventing the appearance of cytopathic effect in 50 % of inoculated cultures was taken as the titer of neutralizing antibody The results obtained are summarized in Table 3

Echovirus was isolated from 5 out of 6 specimens received In the single case in which the CSF was negative (B M), virus was isolated from a TS specimen taken when the patient was seen in the outpatient clinic 6 days before being hospitalized, and from a RS sent from the hospital. Moreover a four-fold rise in neutralizing antibody titer was observed in comparing two serum samples from this patient, taken a month apart. Only a single serum specimen was received from the other patients, but the neutralizing ant body titer was uniformly high, 1 640 and over The TS taken from another child (C. A.) in the out patient clinic yielded virus, and in this case the TS and CSF received from the hospital three days later were also positive

DISCUSSION

Epidemics of aseptic meningitis have been described in recent years by arious uthors. The

agent responsible for a number of them was identified as ECHO 9 virus (2, 3 6 7 12–14 16, 19–24). To date some 30 serotypes of the echovirus group have been isolated and identified (10 18) most of them being implicated, to varying degrees, as causes of aseptic meningitis. Cases with brain damage have rarely been seen, and it is doubtful whether echoviruses cause paralysis in man.

Echoviruses are widely distributed in human populations. Like other enteroviruses, of the Polio and Coxsackie groups, they are prevalent throughout the year in hot climates, and during the summer and autumn months in temperate regions (13 14 22)

Echoviruses are most prevalent among children (1 13 .1 22), particularly in overcrowded populations, where poor facilities and low standards of hygiene are prevalent (8, 11) The particular epidemic, herein described, broke out in a clean middle-class residential quarter with good housing conditions, a fact which may explain its rather limited extent. In cases of outbreaks that were investigated in the past the affected subjects were usually from a limited area, and in many cases more than one member of a family were found to be ill, or to be a carrier of the virus (5 12)

In Israel very few outbreaks, due to infection u echovirus 9 have been reported. Rannon *et al* (21) described an epidemic caused by this virus, in which about 60% of the cases were diagnosed as meningitis. The rest were abortive infections, without meningeal signs. In the outbreak studied by Ashkenazi & Yodfat (1) the salient features were fever sore throat, abdominal pains and vomiting In no case was a meningeal involvement noted.

There is, apparently a great variability in the severity of the symptoms in syndromes accompanying echovirus 9 infections. Only the severe cases are recognized and hospitalized. Most of the affected individuals remain at home (22, 4) suffering from a mild influenza-like syndrome (16) Sometimes other symptoms may appear such as a rash, which occurs mostly in children (12, 15–17 22, 23)

Investigation of the patients famil es and neighbours, in the present outbreak, did not reveal other cases suffering from either a similar syndrome, or milder non-specific febrile syndromes, as has been described in previous reports of such outbreaks. Inquiries revealed a close connection between some of the patients. For example—patients 1 and 2 were brother and sister while patient 3 attended the same kindergarten as patient 1 Patient 5 had a nurse-maid who was a close friend of the parents of patient 4 and used to visit them quite often. It is also noteworthy that the families of all the patients used to buy food in the same grocery and went there almost daily

It may be speculated that, because the only clinical syndrome associated with the outbreak was that of meningitis, a more virulent strain of virus, with greater affinity for the central nervous system, was involved In this connection it may be pertinent to mention that the mouse-pathogenicity of echovirus 9 strains varies greatly some strains being more virulent, causing paralytic disease in suckling mice, while others cause no damage to the animals (4)

The course of the disease was stormy in the beginning. The main symptoms in the first 2–3 days included—severe headache, nuchal rigidity vomiting and in some cases (2, 3 and 5) even—somnolence This was usually accompanied by high temperature (6, 12, 13 16, 22) The maculopapular rash, described by some authors as being a common finding (12, 13 15 17 22, 23), appeared only in one of our patients (No 5)

The findings in the CSF were similar to those noted in the past, in other outbreaks of aseptic meningitis. The typical changes in the fluid included a mild rise in the protein content, up to 50–60 mg/100 ml. A rise above the level of 100 mg/100 ml is only rarely seen (13 22) The cell count varied from 216 to .350 leucocytes per mm³ (in the first stage of the disease), with mononuclear cells predominant. Some authors have reported an initial predominance of polymorphonuclear cells, but later on and in most cases described even in the beginning of the disease mononuclear cells were observed in greater number (12, 12). The rise in cell count in our cases (Table 2) was generally related to the severity of the clinical picture. The milder cases were those with a small elevation of the cell count in the CSF In parallel a fall in the cell count corresponded to improvement in the general condition of the patients.

Electroencephalographic examination was performed in 5 of our patients. In all of them a completely normal pattern was found, even in the

severe cases which were somnolent. An abnormal pattern had been recorded in similar cases, and in some outbreaks it had been observed in nearly 20% of the examined patients (13).

Treatment was based on the administration of symptomatic drugs (antipyretic, analgesic and antiemetic). Children exhibiting somnolence and severe vomiting were given fluids and electrolytes intravenously. Antibiotics—penicillin, streptomycin, chloram-phenicol—were administered to the first two patients only when the aetiology was unclear and a possibility of septic meningitis had to be considered. The last 4 patients to be admitted were treated entirely symptomaticly. There was no difference in the response of the patients to treatment with or without antibiotics.

The duration of the disease was 7–10 days. The children regained regular activity within 1–2 weeks following discharge from the hospital.

SUMMARY

An outbreak of aseptic meningitis broke out in a limited residential area of Jerusalem, Israel, in the early summer of 1967. The patients, whose ages varied from 15 months to 10 years, presented a clinical picture of acute meningitis. Nuchal rigidity positive Kernig's and Brudzinski's signs and high temperature were noted in all cases.

Examination of the CSF revealed pleocytosis, mostly f mononuclear cells.

Echovirus 9 was isolated from the CSF of 5 patients and from throat swab and rectal swab taken from the sixth patient.

The outbreak lasted for 5 weeks, and all the patients recovered completely.

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ELECTROMYOGRAPHIC EVIDENCE OF A MUSCLE LESION IN HOM[illegible]RIA

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A peculiar gait which is variously described as "shuffling" duck-like and "Chaplin-like" has been observed in patients with homocystinuria (1). In order to seek evidence of muscle weakness as a possible factor causing the abnormal gait, electromyography (E.M.G.) was performed in 9 patients with homocystinuria. The findings in them and in an unaffected twin and five parents are described in this paper

CLINICAL FINDINGS

The nine patients, six males and three females, aged twelve months to twenty years, were from six families (three from one family two from another and one from each of four families). They had proven homocystinuria with [illegible] raised serum level of methionine and homocystine. The clinical features are given in Table 1 In all but patients 5 and 6 the gait was abnormal. In two (patients 1 and 7) there was evidence of rather marked pelvic girdle weakness while patients 3, 7 and 8 rose from lying position [illegible] kwardly and needed to use [illegible] climbing up the legs attitude Patients [illegible] young for accurate assessment of muscle strength [illegible] tendon reflexes [illegible] are present, there [illegible] no diff[illegible] muscle wasting and sensation seemed intact There [illegible] no thickening of peripheral nerves. Patients 1, [illegible] and [illegible] are reported in more detail by Carson [illegible] al (1) All patients had [illegible] normal serum creatine phosphokinase value.

ELECTROMYOGRAPHIC STUDY

E.M.G. was carried out on the nine patients, the non-identical twin of patient 4 and in five parents of patients 1, 2, 3 and 4 The deltoid muscle [illegible] as examined b[illegible] concentric needle electrodes 0 65 mm in diameter [illegible] three channelled Disa machine. Restless behaviour [illegible] three patients allowed only [illegible] limited recording but in [illegible] 6 six others and in the sibling and parents, nine different muscle points at three depths, and thus 27 different motor unit territories were sampled as formulated by Buchthal (2). The duration of the motor action potential [illegible] was measured for each position and [illegible] mean value calculated. A value deviating more than 20% from the normal for the muscle in the relevant age group was considered definitely abnormal (7). The standard normal values for this department were established on 48 controls aged 20–60 years sampling the anterior tibial muscle (3), and

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Table 1 *Clinical features*

Limb girdle weakness S severe M mild, ± probable, [illegible] clinical abnormality 0 no clinical abnormality

Case	Family	Sex	Age years	Intelligence quotient	Ectopia lentis	Skeletal abnormality	Cardiovascular involvement	Muscle weakness	Peculiar gait
1	I	M	10	50				S	
2	I	F	7	33 20			●	M	
3	II	M	18	50				–	
4	III	M	8	71			●	0	
5	IV	M	1	82	0	●	●	0	0
6	IV	F	5	93		●		0	0
7	IV	F	10	"6 year level"					
8	V	M	13	37			0		
9	VI	M	20	50				●	–

Table 2. *Summary of results (patients)*

Limb girdle weakness: + + = severe, + = slight, ± = probable, 0 = no, — = value could not be obtained because of lack of co-operation

Case	Family	Sex	Age years	Muscle weakness clinically	Mean duration of all motor action potentials (% increase or decrease)[a]	Incidence polyphasic potentials %	Amplitude of interference pattern (millevolts)
1	I	M	10	+ +	−12	46	—
2	I	F	7	+	−19	14	1 9
3	II	M	18	±	−27	13	1 7
4	III	M	8	0	+ 3 (15)[b]	20	1.5
5	IV	M	1	0	—	0	—
6	IV	F	5	0	− 7 (8)	14	1.0
7	IV	F	10	±	−35 (11)	27	2.5
8	V	M	15	±	−17	15	2.1
9	VI	M	20	0	−26	16	2.1

Deviation from normal value for deltoid muscle in appropriate age group given by Buchthal (1957).
[b] The number of potentials measured, if less than 20, are in parenthesis.

on 56 controls aged 19–74 years examining the deltoid, rectus femoris and abductor digiti minimi muscles (4). In both these studies the same Disa machine and similar needle electrodes (with particular attention given to their checking and maintenance) were used. The standard routine f measuring at least 20 action potentials was followed with each potential identified at least three times and its duration veraged. The same observer examined all the recordings in the present study and the ame criteria for demarcating potentials to allow for liable measurement was rigorously adopted in these ons. The results obtained for the anterior tibial, deltoid, rectus femoris and abducto digiti minimi muscles *for the aproprlate age group fell well within* 20% *devia*tion from the value given by Buchthal, who has also used Disa machine and needle electrodes of type similar to those employed here. Because of this concordance we have adopted Buchthal' normal values for the deltoid muscle in all age groups. The incidence of polyphasic potentials (five phases or more) was deter mined and the uppe limb of normal was taken as 12% (5).

RESULTS

The results are summarized in Table 2 (patients) and Table 3 (relatives) There was no spontaneous activity in the form of fibrillation or fasciculation potentials. There was normal interference pattern but the amplitude of this was in general reduced in the patients and normal in the relatives. The mean duration of the motor unit potential was similar whether polyphasic units were included or excluded. The mean duration value for all potentials is given in the Tables. Reduced duration of motor unit potential greater than 20% was found in 3 patients, while a less significant reduction was present in 4 others. There was an increased incidence in polyphasic potentials in eight patients. In the baby a visual assessment of the potentials was regarded as normal. The duration of action potential was reduced by more than 20% in the

Table 3 *Summary of results (relatives)*

Case	Family	Age years	Mean duration of all motor action potentials (% increase or decrease)[a]	Incidence, polyphasic potentials %	Amplitude of interference pattern (millevolts)
Father	I	32	−29	3	3.6
Mother	I	30	−11	18	2.4
Father	II	58	−22	6	2.7
Father	III	40	−23	14	3.6
Mother	III	33	−15	11	2.3
Non-identical twin sister	III	8	− 9	0	2.4

Deviation from normal value for deltoid muscle in appropriate age group given by Buchthal (1957).

fathers of family I, II and III while the incidence of polyphasic potentials was increased in the father of family III and in the mother of family I. Motor nerve conduction in the median nerve was normal in the three patients in whom it was measured. The latency at the wrist and the conduction velocity from wrist to elbow in the median nerve and the amplitude of the evoked potential with stimulation by ring electrodes of digital nerves, which were performed on one patient were normal.

DISCUSSION

A reduction in the duration of the motor unit potential is currently regarded as a most reliable guide in the diagnosis of dystrophic muscle (6) (7). This was present in 7 of the cases (though in only three was the reduction below 20% of the normal) and together with a higher incidence of polyphasic potentials can be taken as evidence for a myopathic lesion. There was no abnormal insertional activity or spontaneous fibrillation to suggest strongly a process such as polymyositis, but the E.M.G. does not often help in the differentiation from dystrophy. On the basis of the E.M.G. findings a muscle lesion is considered probable which could be myositic but is more likely to be a primary non-inflammatory myopathy.

Adequate histopathological studies are not yet available in the present patients who were all mentally retarded and usually hyperactive. Patient 1 did have a rectus abdominis muscle biopsy which was taken when he was undergoing an operation for inguinal hernia in April 1965. The patient at that time was being investigated by Professor Dent in University College Hospital, London, and the muscle was considered to be normal.

In the autopsy reports of other cases, (8) (9) there is no description of skeletal muscle, while Carson *et al.* (1) state in discussion that muscle obtained at autopsy of their case 2 was normal. By courtesy of Dr. Ingrid Allen, the histology of skeletal muscle (which had been preserved in the Department of Pathology) of a patient (Case 1) reported by Gibson *et al.* (8) was reviewed and was considered to be normal although the small blood vessels supplying the muscle were thickened and fibrous but not thrombosed.

The E.M.G. in the fathers of families I, II and III was abnormal with more than J reduction in the duration of motor action potentials. These parents were clinically normal and had no homocystinuria and a normal value of serum creatine phosphokinase. In muscular dystrophy the E.M.G. is not usually a satisfactory way of diagnosing the unaffected carrier (10). The abnormal E.M.G. in the parents may indicate that they are heterozygotes for the disease which appears to have an autosomal recessive mode of inheritance. So far in the Northern Ireland series, homocystinuria has not been detected in non-affected relatives. Laster *et al.* (11) report only one such occurrence and the only other known means of diagnosing the heterozygote is by determining a lower value of cystathionine synthetase in liver biopsy material of unaffected relatives (12) (13). Therefore, one should consider further studies of muscle as a means of identifying the heterozygote.

Brief mention could be made of the factors which might produce a muscle lesion in homocystinuria. Thomas (14) has found no myopathic changes in the E.M.G. in "hypotonic" cerebral palsy and it is felt that brain disease *per se* would not produce the E.M.G. alterations observed in the present cases. Intravascular clotting associated with increased platelet stickiness is a feature of homocystinuria (15) and could conceivably contribute to ischaemic muscle dysfunction. An important factor however would appear to be the metabolic abnormality itself. Brenton *et al.* (16) have emphasized that cystine may be considered an essential amino acid in homocystinuria. If cystine were deficient in the diet in such patients or in relationship to malabsorption across the gut (steatorrhoea is described by Gibson *et al.* (8) in one case) then changes in the muscle akin to experimental nutritional muscular dystrophy might occur (17). Finally the association may be based on homocystinuria affecting organs having a common mesodermal origin. Such an explanation is offered by Capotorti (18) for the occurrence of congenital muscular dystrophy in a case of Marfan's syndrome.

It is not suggested in this paper that muscle weakness is the sole or main cause of the peculiar gait. Skeletal abnormalities (16), as well as poor intellectual development must be of great importance and one would agree with Dubowitz (19) that "all that waddles is not dystrophy". However

It is concluded that there is a muscle lesion in homocystinuria which is probably myopathic.

SUMMARY

In seven out of nine patients with homocystinuria, electromyographic examination suggested a myopathy. The E.M.G. was also abnormal and of myopathic type in three of the parents. The implication of this finding in homocystinuria and in unaffected relatives is discussed. It is concluded that there is a muscle lesion in homocystinuria which is probably myopathic and is one factor producing the peculiar gait present in this disease.

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POLIOVIRUS ANTIBODY STATUS AND THE RESPONSE TO TRIVAL ORAL POLIOVIRUS VACCINE IN NORWEGIAN SCHOOL CHILDRE

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The inactivated poliovirus vaccine (Salk) has been administered in Norway since 1956. Trivalent oral poliovirus vaccine (OPV) was introduced in the autumn of 1965.

The following groups were initially recommended for vaccination with OPV: 1) Infants and pre-school children previously not vaccinated with Salk vaccine and 2) school-children in their first and last year at the elementary school (usually 7 and 14 years of age), regardless of previous immunization with Salk vaccine. Three doses of OPV were administered, the intervals between doses being about six weeks.

An investigation of the antibody status among a group of seven years old Norwegian school children immediately before the introduction of OPV is described in the first part of this report. Some of the children had received a full course of Salk vaccine while some of them had not been given any poliovirus vaccine. The second part of the report is concerned with the antibody response to OPV in these children.

Older siblings to children given OPV who had received no oral vaccine were included in order to study the possible spread of vaccine strains to close contacts.

PLAN OF THE TRIAL

A total of 1 4 children from five different schools in Oslo was included in the study. Eighty of the children were in their first year at school. They were about seven years old. Forty-six of these children had received no vaccine before school entry (group I). The remaining 34 children were previously Salk vaccinated (group II). The first two injections of vaccine had been given at six to eight months of age, the third seven months later and the fourth injection was given at the age of ... or three years. A few of them had not received the fourth injection.

All children in groups I and II received three doses of OPV at intervals of about six weeks starting in October 1965.

Forty-four other school children were also studied. They were older siblings of children receiving OPV ranging in age from 8 to 14 years (group III). All of them were Salk vaccinated. The age of these children at the time of vaccination varied considerably. The first injection had been administered from one to five years before the present investigation.

Blood samples were collected from all the 1 4 children before and about 4 weeks after completed oral vaccination of the children in groups I and II.

Table 1 shows the population studied in relation to previous and present poliovirus vaccination.

The number of children in groups I and II was the result of an initial effort to obtain an adequate number of non-Salk vaccinated children. According to the school health authorities in Oslo about ten per cent of the children who entered school had received no Salk vaccine.

MATERIALS AND METHODS

Vaccines

Inactivated poliovirus vaccine manufactured in the United States was used from 1956 onwards. In 1956–7 Danish produced vaccine was also administered.

The vaccine was administered by intracutaneous injections in 1956–7 and since 1958 by subcutaneous injections.

Trivalent oral poliovirus vaccine (OPV) from Glaxo Laboratories Ltd. was used from 1965. The vaccine is prepared from Sabin's live attenuated strains of poliovirus. According to the manufacturer one dose of vaccine contains 10[illegible] T.C.I.D.50 of type 1 virus, 10[illegible] T.C.I.D.50 of type 2, and 10[illegible] T.C.I.D.50 of type 3.

Blood Sampling and Serological Test

The blood samples were taken by fingerprick and each sample was collected on three filter paper disks as described by Brady et al. (2). The disks were allowed to dry at room temperature for some days and then stored

Table 1. *Previous and present poliovirus vaccination of the children studied*

		Previously Salk vaccinated		
		No	Yes	Total
Received OPV in present trial	Yes	46[a] (group I)	34 (group II)	80
	No		44 (group III)	44
Total		46	78	1.4

No. f children

in rubber stoppered glass tubes at −20°C until tested. The serum from the three disks was eluted in one ml of phosphate buffered saline at +4°C for 18 hours. The disks were then squeezed with forceps and discarded. The eluate was inactivated at 56°C for 30 minutes, centrifuged and immediately examined in the neutralization test. Preliminary tests were performed t determine the concentration of serum in eluat Protein determination as well as comparative neutralization tests on eluate and venous blood drawn from the same person showed that the eluate contained approximately one to ten dilution of the corresponding serum

The neutralization test was performed in secondary cynomolgus monkey kidney cell cultures in tubes. Serial tw fold dilutions of the sera ranging from 1 10 to 1 2560 were tested against approximately 100 T.C.I.D.50 poliovirus type 1 (Brunhilde), type 2 (Lansing) and type 3 (Leon). The serum-virus mixtures were allowed to combine for one hour at 37°C. Due to the small amounts of eluate available only one cell culture tube was inoculated per serum-virus mixture The tw eluates from each child were always tested simultaneously The antibody titre is expressed as the reciprocal of the highest dilution of serum giving complete neutralization of the virus.

The British standard poliomyelitis antisera (8) were also titrated as outlined abov According to these titrations antibody detectable only at 1 10 dilution expressed in International units (6) were as follows: For type 1, 0.8 I.U. for type 2, 0.4 I.U. and for type 3 0.9 I.U. per ml.

RESULTS

Antibody Status before Vaccination with OPV

Group I The distribution of detectable antibodies to the three types of poliovirus in the first blood samples is shown in Table 2. The proportion of children without antibodies is strikingly high in this group Thirty-seven or 80 per cent lacked antibody to all three types of poliovirus (triple negative) Fifteen per cent had antibodies against type 1

Groups II and III The children in group II and in group III had all received Salk vaccine and are here considered together As can be seen in Table 2, the figures for the two groups do not differ very much.

Compared with group I, antibodies were detected more often among these 78 children. Forty one per cent had detectable antibodies against type 1 while 76 per cent had antibodies against type 2 and 56 per cent had antibodies against type 3 (Table 2). Twelve per cent were triple negative. Antibodies against poliovirus type 1 appeared more often among children who had received their last injection of Salk vaccine not later than three years ago. No such correlation could be demonstrated for types 2 and 3

Antibody Response after Vaccination with OPV

Group I and group II The antibody response among vaccinees without detectable antibody

Table 2. *Antibody status before vaccination with OPV*

Antibody against poliovirus	Group I	Group II	Group III	Groups II and III
None	37[b]	6	5	11
All three types	0	11	12	23
Type 1	7	12	20	32
Type 2	4	26	33	59
Type 3	2	22	22	44
No. of children in the group	46	34	44	78

Antibody detectable in serum dilution 1 10 or more
[b] No. of children

Table 3. *Antibody response to oral poliovirus vaccine in children of group I and group II*

Group of children	Polio-virus type	No. of children without detectable antibody: Before oral vaccination	After oral vaccination	Conversion rate in %
I	1	39	6	33/39 84
	2	42	1	41/42 97
	3	44	8	36/44 81
II	1	22	0	22/22 = 100
	2	8	0	8/8 100
	3	12	1	11/12 = 91

Table 4. *Postvaccination antibody titres among children in groups I and II where the pre-vaccination sera were without detectable antibody against the corresponding virus type*

Polio-virus type	Group of children	Antibody titre after oral vaccination <10[a]	10	20	40	80	160	320	640	1280	>2560	Total
1	I	6	7	6	7	6	4	2	1	0	0	39
	II	0	1	0	3	4	8	5	1	0	0	22
2	I	1	0	5	6	8	11	7	2	2	0	42
	II	0	0	0	1	0	1	2	2	1	1	8
3	I	8	6	12	6	6	4	1	0	1	0	44
	II	1	0	3	4	1	1	2	0	0	0	12

Reciprocal of serum dilution
No. of children

against the corresponding type in the first blood sample is shown in Table 3. It will be seen that the conversion rate was almost 100 per cent for the children in group II, and somewhat lower for group I. The number of triple positive children were 32 in group I and 33 in group II or 69 and 97 per cent respectively. The effect of the oral vaccine may also be illustrated by the following figures: Thirty of the 43 initially triple negative children developed antibodies against all three polio-virus types, and none remained triple negative.

We have compared the antibody response of children in group I and group II, who were without detectable antibody to the corresponding type in their first blood sample. The antibody titres in the second blood samples are shown in Table 4. There was a tendency for children previously Salk vaccinated to respond with higher antibody titres. The difference in response was highly significant for poliovirus type 1 ($p < 0.001$) and type 2 ($p < 0.01$) but not for type 3 ($0.15 > p > 0.10$) (Two-sample rank test.)

Group III. Twenty-four of the children in this group were without detectable antibodies against poliovirus type 1 in their first blood sample. Seven of them had developed antibodies after completed vaccination of their sibling. Correspondingly six of eleven initially sero-negative children developed antibodies against type 2 and three of twenty-two initially sero-negative children developed antibodies against type 3.

DISCUSSION

The seven years old children were all born after the introduction of the Salk vaccine in Norway. It will be seen that among the children in group I, none of whom had received Salk vaccine, there were only 20 per cent with antibodies against one or more poliovirus types. Serological investigations among Scandinavian children of the same age group before the introduction of Salk vaccine all showed a much greater prevalence of neutralizing antibodies (5, 7, 11). Although direct comparison is difficult due to different laboratory techniques, the results do indicate a decrease in natural infections with poliovirus after the introduction of Salk vaccine.

Among the 78 children in groups II and III, all of whom had received Salk vaccine, only 41 per cent had demonstrable antibodies against type 1 and 76 per cent against type 2, whereas 56 per cent had antibodies against type 3. The figures are rather low as compared with other long time surveys of Salk immunized children (3). This may in part be due to our somewhat inaccurate neutralization method, starting with serum dilution of one to ten and incubating the serum-virus mixture for only one hour. However titration of the British standard sera revealed that our procedure was moderately sensitive. Furthermore, the lack of detectable antibodies need not mean that the Salk vaccine was ineffective. Thus initially seronegative children in group II compared with group I children responded with greater antibody rise when fed OPV (Table 4). This may indicate a secondary response in a sensitized subject as has been suggested by Beale *et al.* (1).

The results suggest however that most of the children who reached school age without any immunization against poliomyelitis and also a considerable number of the previously Salk-immunized children were without proper antibody protection.

Similar findings have been reported from Great Britain by Skelton *et al* (10) It thus seemed necessary to vaccinate or revaccinate all school children against poliomyelitis. It should be added that in 1966–67 all children and young adults in Norway have been offered oral poliovirus vaccine regardless of earlier immunization with the Salk vaccine.

The response to the oral vaccine as measured by conversion rates for the three virus types varied between 81 and 100 per cent. The results for group II agree with those of other similar investigations (9) while the response of the children in group I seems to be less satisfying (Table 3) The difference in seroconversion rate is probably due to the difference in antibody status before oral vaccination, a much greater proportion of the children in group I being, initially triple negative than in group II (Table 2) A kind of secondary response to OPV among some of the Salk immunized children (see above) may also favour the better effect among the children in this group

The highest conversion rate for all vaccinees was found for poliovirus type 2 in accordance with earlier observations that this type is the most active component of Sabin's trivalent vaccine (4)

The conversion rates among children in group seem to indicate that types 1 and 2, possibly type 3 of the vaccine strains were transmitted close contacts.

SUMMARY

Neutralizing antibodies against poliovirus were studied among 124 Norwegian school children, 80 of whom received three doses of trivalent oral poliovirus vaccine during the study the remaining 44 were older siblings to vaccinees.

Eighty per cent of 46 children with no previous Salk immunization lacked demonstrable antibodies against all three types of poliovirus at school entry Among previously Salk vaccinated children, a considerable number seemed to be without antibody protection against type 1 and type 3

Antibody response to oral vaccine as measured by sero-conversion ranged between 81 and 100 per cent.

Spread of vaccine strains to older siblings was demonstrated serologically

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DIETARY TREATMENT OF CYSTINOSIS

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Cystinosis is a hereditary metabolic disorder probably due to a defect in the normal degradation of cystine, leading to cystine storage in most organs and tissues (16). The exact nature of the defect is not known. The clinical picture is in the initial stages of the disease dominated by renal tubular damage, leading to defective reabsorption of water phosphate, glucose, potassium, bicarbonate, amino acids etc. The symptoms usually start at four to six months of age. Polyuria, thirst, anorexia, vomiting, weight loss and growth failure are noticed. Vitamin D resistant rickets is common. Photophobia due to deposition of cystine crystals in the cornea is frequently found. Soon signs of more generalized renal failure with impairment also of glomerular function develop. Death may occur in electrolyte crises in the early stages of the disease, but more often, following years of illness, from renal failure or from infection. Adult age is rarely if ever reached. More detailed discussions of the different aspects of the disease may be found in several recent publications (3 4 6, 13 14 19)

Until recently treatment with relatively high doses of vitamin D and with solutions of sodium citrate, potassium citrate and citric acid have mostly been used. A temporary improvement of the clinical situation can thereby be obtained. Penicillamine has been tried (2, 19), but does not seem to be effective. Treatment with anabolic steroids has been reported to be of some value, at least temporarily (23)

A more promising approach to therapy should theoretically be to give a diet low in methionine, cysteine and cystine, in an attempt to reduce the storage of cystine in the tissues and, if possible, mobilize the cystine deposits. (Methionine and cysteine are to great extent metabolized to cystine in the body.) At least one favorable report along these lines has been published (1). A special lentils diet was used in this case. A diet prepared from casein hydrolysate has also been proposed (15), but so far no clinical results have been reported.

We have in the Department of Pediatrics, Rikshospitalet, University of Oslo studied two brothers suffering from cystinosis. They have been treated with the lentils diet for the last 8 months The preliminary results are promising and will be reported below The results of quantitative estimations of the amino acids in serum urine, spinal fluid, and liver before and during therapy will also be presented.

METHODS

Amino acid determinations were performed using Technicon Amino Acid Autoanalyzer The column (140 cm by 0 62 cm) equipped with Type A Chromobeads (8% crosslinked spherical particles and having specified diameter of 18–25 μ) was used in this study A 9-chamber Autograd with citrate buffers gave gradient of pH from 2.875 to 5.00 (22). The flow rate was 30 ml hr and the chromatogram was completed in 21 hrs. The column was first operated at 37°C After 2.5 hrs the temperature was raised to 60°C, and this temperature was maintained for the rest of the analyses.

Preparation and application of samples to the column (9)

The samples of urine, serum, cerebrospinal fluid and liver were stored at −25°C until analyses.

Abbreviations: PEA = Phosphoethanolamine, Tau = taurine, Hyp = hydroxyproline, MSO = methionine sulfoxide, Asp = aspartic acid, MSO_2 = methionine sulfone, Thr = threonine, Ser = serine, Glu glutamic acid, Pro = proline, Cit = citrulline, Gly = glycine, Ala = alanine, Abu = α-amino-n-butyric acid, Val = valine, CSS cystine, Met = methionine, Cth = cystathionine, Ile = isoleucine, Leu = leucine, Tyr = tyrosine, Phe phenylalanine, Baib = β-aminoisobutyric acid, Eth = ethanolamine, Hyl = hydroxylysine, Dbu = 2,4-diaminobutyric acid, Orn ornithine, Lys = lysine 1 Meh 1 methylhistidine, His histidine, 3 Meh = 3 methylhistidine, Car = carnosine and Arg = arginine.



Fig 1 Cystine crystals in the bone marrow

Urine Urine and serum were deproteinized before analysis. 4 ml of urine as treated ith 150 mg of solid sulfosalicylic acid and the mixture was shaken and centrifuged. An amount of supernatant equivalent to 100 µg of creatinine f children and 400 µg of creatinine for adults was applied directly to the column, followed by 0.1 ml of standard solution containing 0.25 µmole of norleucine

Serum. 2 ml of serum as transferred to centrifuge tube, and the protein was precipitated by the addition of 100 mg of solid sulfosalicylic acid After centrifugation, 0.5 ml of the supernatant was pplied directly to the column followed by the norleucine internal standard

Cerebrospinal fluid. Cerebrospinal fluid was not deproteinized before analysis. 2 ml of the CSF sample was applied directly to the column together with 0 2 ml f 1.0 N hydrochloric acid and the norleucine internal standard.

Liver 400 mg of liver was homogenized in 96% ethylalcohol and the alcoholic-extract was evaporated to dryness at 40°C in stream of air The residue was taken up in 1 ml of 0.1 N hydrochloric acid. After centrifugation, 0.5 ml of the supernatant as applied directly to the column followed by internal standard.

Diet 50 mg of lentils diet was examined on free amino acids.

CASE REPORTS

Case 1

A. K. is boy born March 23 1962 following normal pregnancy and delivery as the first child of healthy unrelated parents. Birth weight 3630 g, length 51 cm. He developed normally until about six months of age, when he began to refuse taking solid foods. Polydipsia, polyuria and constipation were noticed, and soon also growth retardation. Salt hunger has been prominent feature

At 2 /ₘ years of age he was seen in our department for the first time. His height was 78 cm, i.e. 5 cm below the 2.5 percentile, weight 9450 g. No radiological sign of rickets.

H was readmitted to the department at 5 years of age in order to be evaluated for dietary treatment. His height was now 90.5 cm, i.e. 13 cm below the 2.5 percentile Weight 13.3 kg. Bone age was 2 /ₘ years. There was obvious photophobia. Numerous cystine crystals were present in the cornea and in the bone marrow (Fig. 1) Liver biopsy showed deposits of cystine in the periportal tissue and in occasional liver cells, and there was mild increase in periportal connective tissue. He was drinking between 2.5 and 3 liters of fluids/24 hours and usually awake three or four times during the night in order to drink water or juice. The results of some laboratory studies are presented in Table 1.

Table 1 *Laboratory data before dietary treatment*

	Case 1		Case 2,
	1964	1967[a]	1967[a]
Proteinuria	+	+	+
Glucosuria	+	+	+
Generalized hyper aminoaciduria	–	+	+
Hemoglobin, g/100 ml	10.2	11.5	11 1
Blood urea, mg/100 ml	69–89	97	120
Creatinine, mg/100 ml		1 7	1.9
Potassium, mEq/l	2.9–3.7	4.2	3.8
Sodium, mEq/l	130–139	132	143
Chlorides, mEq/l	97 104	91	93
Calcium, mEq/l	5.0	4 9	5 7
Phosphorus, mg/100 ml	3.2	5.0	5.5
Alkaline phosphatase, Bodansky U	16 0	11.2	9.4
Standard bicarbonate	15	21	23
pH	7.24–7.35	7.37	7 40

On treatment ith vitamin D, 14 000 I.U daily and solution of sodium citrate, potassium citrate, and citric acid.

Case 2

T K., the younger brother was born at term on Nov 26, 1963 by caesarean section due to placenta praevia. Birth eight 3520 g, length 51 cm. The progress of the disease has been almost exactly like in his older brother At 3 /12 years of age his height was 83 cm, i.e. 8 cm below the 2.5 percentile. Weight 12.4 kg. Laboratory data: see Table 1

TREATMENT

Until the dietary treatment was started, both patients received vitamin D 14 000 I U daily and a solution of sodium citrate, potassium citrate and citric acid. This medication was continued unchanged during the period of dietary treatment.

The diet low in methionine and cystine/cysteine was started on April 4 1967 A special lentils diet prepared by A/S Nestlé has been used as the main source of protein, essentially in the way described by Bauer & Antener (1) Otherwise the diet is composed of gluten-free bread, margarine, vegetables, fruits, and glucose. Choline chloride in an amount of 0.5 g daily has been given a methyl donor No anabolic steroids have been given.

With this dietary regimen the intake of S-containing amino acids can be reduced to about 30 mg/kg/24 hrs, namely about 20 mg/kg of methionine and 10 mg/kg of cystine (1). The daily protein intake is calculated to be 26.3 g, or about g/kg body weight.

The lentils diet has an accep aste, and there have not been too large pr with the dietary regimen. The mother of t ents is a nurse with good understanding of portance of keeping a strict diet, and she een able to administer it at home.

The most striking effect of the treatment has been a diminution of fluid intake and diuresis. This was observed already during the fir t weeks of therapy Before the diet was started both patients were awake three or four times every night to drink water or juice and to change diaper and the fluid intake was 2500–3000 ml 4 hours On the dietary regimen they are awake once and sometimes not at all during the nighttime The daily fluid intake is about 1500–1650 ml and the diuresis 1400–1650 ml. The patients appetite has improved and is now normal. There has been a weight gain of 0.4 and 1_ kg respectively n eight months. They are more active and vigorous than before. The rate of growth has remained essentially unchanged. It is still too early to say whether it will be possible to mobilize the cystine deposits by this restriction of methionine and cystine intake

As regards the laboratory findings, the most important change has occurred in the blood urea, which has decreased in both patients, from 97 and 120 mg/100 ml, respectively to 33–43 and 58–68 mg/100 ml. The fall in blood urea was observed already after two to three weeks on diet The serum creatinine has remained unchanged so far The pecific gravity of the urine varied between 1.002 and 1 006 before the dietary treatment was started. In the older brother a concentration test was performed, and the maximal specific gra ity of the urine under the test was 1.006 On diet values up to 1 017 have been observed in the older and up to 1 015 in the younger brother Small amounts of protein and glucose are still present in the urine. The serum electrolytes and acid-base status remain essentially w thin normal limits.

AMINO ACID DETERMINATIONS

The results of the amino acid determinations on the two children are shown n the Tables 2–5 Tables 2 and 3 show the amino acids in the urine and in the serum. Four samples of urine and four samples of serum from each child, one before and three after dietary treatment were nalysed.

Table 2. *Urinary amino acids in cystinosis (μ moles/mg creatinine) before (4.22.67) and during dietary treatment*

	T. K.				A. K.				
Amino acid	4.22.67	5.8.67	6.15.67	8.24.67	4.22.67	5.8.67	6.15.67	8.24.67	Normal children[a]
Tau	0.44	0.67	(*)	0.23	0.63	Trace	1.74	0.36	0.38–0.59
Hyp	1.96	(*)	(*)	(*)	1.95	(*)	(*)	(*)	0
MSO	0	0	0	0	0	0	0	0	0.11–0.25
Asp	0	0	0	0	0	0	0	0	0.02–0.03
MSO_2	0.54	1.32	(*)	(*)	0.43	1.52	1.23	1.30	0
Thr	0.14	0.66	(*)	(*)	Trace	0.43	(*)	(*)	0.06–0.13
Ser	(*)	(*)	(*)	(*)	Trace	Trace	(*)	(*)	0.37–0.59
Glu	0.58	4.30	4.33	(*)	(*)	6.03	2.62	0.29	0.03–0.04
Pro	2.70	(*)	4.88	4.04	5.00	(*)	2.87	3.23	Trace
Cit	1.68	1.01	2.53	2.14	1.31	1.11	1.36	3.06	0.01–0.03
Gly	7.58	4.57	14.41	11.32	7.09	5.77	10.80	17.19	0.59–1.31
Ala	11.89	3.19	14.62	7.40	7.70	5.25	3.60	8.48	0.12–0.27
Abu	0.28	0.13	0.21	0.11	0.26	0.16	0.29	0.23	0.01–0.02
Val	2.68	0.82	.41	0.09	3.08	1.04	1.67	1.16	0.07–0.10
CSS	0.31	1.39	2.34	0	0	Trace	3.05	0	0.06–0.08
Met	0.29	0	0	0	0.12	0	0.19	0	0.01–0.02
Cth	0.21	Trace	Trace	0	0.09	Trace	Trace	0	0.10
Ile	0.06	Trace	0.42	Trace	0.24	Trace	0.24	Trace	0.01–0.03
Leu	0.31	0.13	0.61	Trace	0.59	Trace	0.69	0.07	0.13–0.17
Tyr	Trace	Trace	0.75	0.09	Trace	0.12	1.10	0.05	0.08
Phe	0.81	0.47	0.94	0.08	0.88	0.59	0.75	0.59	0.05–0.14
Balb	0.42	(*)	(*)	0.44	1.36	1.13	2.77	0.94	0.05–1.86
Eth	0	(*)	0	0	0	(*)	0	0	0.29–0.67
Hyl	Trace	Trace	(*)	(*)	Trace	Trace	Trace	(*)	(*)
Dbu	Trace	0.12	Trace	0.43	(*)	0.19	Trace	0.21	0.07–0.09
Orn	0.41	0.20	1.80	0	0.19	0.12	0.60	0	0.10
Lys, 1 MeH	2.70	1.43	3.39	0.53	2.21	1.37	2.39	1.41	0.36–0.47
His, M H	1.53	1.36	(*)	0.80	1.40	0.92	1.90	2.43	0.60–0.83
	0	0		Trace	0	0	0	Trace	0.04–0.21
		0.38		(*)	Trace	Trace		0.27	0.04–0.05

[a] Age 2 5/12, 2 8/12 and 5 4/12

[*] Not measured due to insufficient separation.

The urinary amino acids are compared with values obtained from 3 normal children investigated with the same equipment. The amino acids in serum are compared with the normal plasma values reported by Hagge & Brodehl (12). In addition to the amino acids shown in Tables 2 and 3 the chromatograms on diet show sarcosine, canavanine and some unidentified peaks which are of dietary origin, since they also were present in the diet.

The amounts of amino acids in the cerebrospinal fluid and the liver are given in Tables 4 and 5. The tables show only one cerebrospinal fluid sample and one liver sample taken from A. K. after dietary treatment. These values are compared with literature references (8, 14).

Both children have, before dietary treatment, *an aminoaciduria* with the same pattern as seen in the Fanconi syndrome (11, 17). More than 10 amino acids are excreted in increased amounts, namely hydroxyproline, methionine sulfone, glutamic acid, proline, citrulline, glycine, alanine, α-amino-*n*-butyric acid, valine, cystine (T. K.), methionine, isoleucine, leucine, phenylalanine, ornithine, lysine + 1 methylhistidine and histidine + 3 methylhistidine, while the excretion of methionine sulfoxide, cystine (A. K.), aspartic acid, tyrosine, ethanolamine and carnosine are reduced.

The changes in *the urinary amino acids* were not dramatic after dietary treatment. There was no excretion of cystine, methionine, cystathionine and ornithine in the urine. The excretion of proline (A. K.), α-amino-*n*-butyric acid, valine, isoleucine, leucine, phenylalanine, lysine + 1 methylhistidine and histidine + 3 methylhistidine (T. K.) also decreased after dietary treatment, whereas the excretion of methionine sulfone, proline (T. K.),

Table 3. *Serum amino acids in cystinosis (μ moles/l serum) before (4.22.67) and during diet treatment*

Amino acid	T. K. 4.22.67	T. K. 5.8.67	T. K. 6.10.67	T. K. 8.23.67	A. K. 4.22.67	A. K. 5.8.67	A. K. 6.10.67	A. K. 8.23	Normal children[a]
Tau	153	175	134	139	100	142	100	118	57
MSO	13	35	14	10	14	10	43	10	
Asp	0	(*)	(*)	(*)	(*)	(*)	(*)	39	1
Thr	132	(*)	(*)	(*)	79	77	90	116	
Ser	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	
Glu	175	185	202	194	156	163	160	156	136–[illegible]
Pro	315	(*)	(*)	181	(*)	(*)	(*)	166	88–131
Cit	87	82	83	80	66	53	64	78	
Gly	405	332	341	546	412	313	392	595	146–254
Ala	441	382	366	643	438	322	395	562	149–[illegible]1
Abu	16	11	14	9	20	19	16	1	
Val	204	169	158	177	226	178	143	142	164–181
CSS	14	34	17	19	29	25	13	20	43
Met	25	22	22	15	17	20	20	20	11 14
Ile	59	42	42	47	61	52	34	32	42–4
Leu	126	107	87	95	127	110	76	68	81 106
Tyr	50	43	53	60	44	37	37	40	36–64
Phe	114	114	89	105	112	95	80	84	41 54
Orn	88	71	150	127	67	60	99	92	80
Lys	169	158	173	135	161	132	159	136	148–167
His	87	93	92	101	94	83	78	98	99–113
Arg	246	162	143	124	127	110	119	129	107 123

[a] Values of Hagge & Brodehl (12).
[b] Not measured due to insufficient separation.

citrulline, glycine, tyrosine, histidine + 3 methylhistidine (A. K.) and carnosine increased. The other urinary amino acids were unchanged after dietary treatment.

Table 3 shows that the concentration of *the serum amino acids* has less deviation from the normal values than the urinary amino acids. Before the dietary treatment, however there is an increase of taurine, proline (T K.) citrulline, glycine, alanine, valine, methionine, isoleucine, leucine, phenylalanine and arginine (T K.). The cystine values were too low all the time, probably due to the binding of cystine to the serum protein which occurs when protein is not precipitated and removed before deep freezing (21).

After dietary treatment the concentrations of proline (T K.) α-amino-n-butyric acid, valine, isoleucine, leucine, phenylalanine, lysine + 3 methylhistidine, and arginine (T K.) in the serum are smaller than before the dietary treatment. There is an increased concentration of glycine alanine and ornithine, after the treatment, while the concentration of the other amino acids is unchanged.

The cerebrospinal fluid see Table 4 shows high content of citrulline and cystine together with

Table 4 *Cerebrospinal fluid amino acids in cystinosis* μ moles/l

Amino acid	A. K.	Mean[a]	Standard deviation ±
PEA	8.5		
Tau	3.3	6.3	1.8
MSO	2 6		
Asp		0 9	0.5
Thr	14.3	24 8	10.1
Ser	(b)	37 8	22.9
Glu	3 1	7 0	4.9
Pro		0 6	1 6
Cit	3.8	2.0	0.8
Gly	6.6	6 6	1.8
Ala	21 1	23.2	9 4
Abu	1.8	3.4	1.9
Val	7 4	14 6	5.5
CSS	1.0	0.2	0.3
Met	2.2	2 6	1.6
Ile	2.4	4.4	1.3
Leu	5 1	10 9	3.6
Tyr	5.9	9 1	5.0
Phe	7 6	9 2	5.8
Eth	5.3	12.8	5 7
Orn	5.2	5 7	1 8
Lys	10.3	18 7	6.6
His	12.3	13.0	4 4
Arg	23.3	20.1	5.8

Values of Dickinson & Hamilton (8).
Not measured due to insufficient separation.

Table 5 *Liver amino acids in cystinosis*

μ moles/g

Amino acid	A K.	Normal male 1 3/12 years[a]	Cystinosis
PEA	0 92		
Tau	0.43	0.53	0.54–0 69
MSO	0.63	0.41	Trace–0.18
Asp	0.85	0.71	Trace–0.31
Thr	(*b*)	0.54	0.34–1 63
Ser	(*b*)	0 08	0.06–1 40
Glu	3 97	2.39	43–4.96
Pro	(*b*)		0.05–0.23
Cit	0.08		
Gly	2.78	1.56	2.77–4.39
Ala	2.69	4 42	84–2.92
Abu	?		
Val	?	0.10	
CSS	5.58	0.34	4 50–12.57
Met	?	0 03	0.12–0.20
Ile	0.09	0.08	0.14–0 54
Leu	0.22	0 19	0.18–1 14
Tyr	0.12	0 08	0.09–0 29
Phe	0 12	0.04	0.11–0.39
Eth	1 92		
Orn	0.37		
Lys	0.35	0.38	0 45–1.39
His	0.43	0.32	0.28–0.43
Arg	0.05	0.01	0

Values of Linneweh (14).
[b] Not measured due to insufficient separation.

…w content of taurine, valine isoleucine, leucine, …lanine and lysine.

The liver tissue contains increased amounts of glycine, cystine and phenylalanine, while the alanine value is too low As one can see in Table 5 the pattern of these aminoacids is in good accordance with the values found by Linneweh (14).

The free amino acids in the urine and the serum from the parents were all within the normal range. Schneider *et al* (20) have, however recently reported that in patients with cystinosis, the concentration of free cystine in leukocytes is 80 times greater than normal, and six times the normal content in their parents.

DISCUSSION

Dietary restriction of methionine and cystine did not profoundly affect the amino acids in the urine and the serum. But one might emphasize that the changes of the amino acids both in the urine and the serum show a slight tendency towards normalization. It is, however difficult to say whether these changes in the amino acids, are due to the reduced protein intake or to the reduced amounts of methionine and cystine in the diet *per se*

As far as the S-containing amino acids is concerned, the urinary excretion of cystine, methionine and cystathionine decreased after dietary treatment and the last urinary samples did not show these amino acids at all. The excretion of taurine, however changed very little and the methionine sulf oxide remained absent. The elevated excretion of methionine sulfone in the urine increased after the dietary treatment. The serum concentration of taurine, methionine sulfoxide, cystine and methionine was about the same after dietary treatment.

As described above, there has been an obvious improvement in the *clinical condition* of the patients during the dietary regimen, with increased well-being, reduction in fluid intake and diuresis, and fall in blood urea, but no improvement in the rate of growth.

In this connection two questions can be raised. Could the observed improvement possibly be due only to the reduction in protein intake, and not a specific effect of the reduced intake of S-containing amino acids with real improvement of the cystinosis? And will it be possible by prolonged dietary treatment to mobilize cystine from the deposits, prevent further deposition, and thereby prevent the fatal outcome of the disease?

The latter question can not be answered as yet, although the experiences of Bauer & Antener (1) in one case, and our own experiences certainly justify that further attempts with long-term dietary treatment are made.

When trying to answer the first question, it should be remembered that the lentils diet given, containing about 26 g of protein per day is not particularly low in protein. However approximative calculations of the patients protein intakes before the diet was started, indicate that the daily intakes have been about 40 g, perhaps slightly higher The reduction in protein intake *per se* would tend to lower the blood urea, and thereby possibly alleviate some of the patients symptoms and signs. But it is in our opinion not very likely that this is the whole explanation of the favorable course. The blood urea has dropped to about half the pretreatment level, even though the diuresis is about 1000 ml lower than before therapy The increase in specific gravity of the urine on the diet

indicates that the capacity of the kidneys to concentrate the urine (i.e. reabsorbe water) has in fact improved. Earlier attempts to treat cystinosis with simple reduction of the protein intake (1) do not seem to have been successful, either.

SUMMARY

The amino acid pattern in the urine and serum was investigated in two cases of cystinosis before and on treatment with a diet low in methionine and cystine. The cerebrospinal fluid and the liver were studied two months after dietary treatment was started. The urinary amino acids in both cases were increased, similarly as in the Fanconi syndrome. Some of the amino acids occurred in elevated amounts in serum. The aminoaciduria, therefore, must be extrarenal as well as renal in origin. The treatment with the methionine and cystine poor diet did not produce a great change in the concentration of the urinary and serum amino acids, but the amino acids showed a slight tendency towards normalization. The cerebrospinal fluid and the liver showed high levels of cystine.

Clinically there was an obvious improvement on the dietary regimen with increased well-being, reduction of fluid intake and diuresis as well as a fall in blood urea and an increase in specific gravity of the urine. The rate of growth has been unchanged so far.

Whether all these positive findings are due to the reduced protein intake or to the reduced amounts of methionine and cystine in the diet, is difficult to decide at the present time.

ACKNOWLEDGEMENTS

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METHYLMALONIC ACIDEMIA

A Disorder Associated with Acidosis, Hyperglycinemia, and Hyperlactatemia

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Methylmalonic aciduria (18) or methylmalonic acidemia (20) is a newly described inborn error of metabolism. The clinical features of the three cases reported until now have been vomiting, failure to thrive and persistent mild to moderate acidosis. In association with mild infections acute life-threatening crises of acidosis have appeared One of the cases which has been reported (18) was alive and normally developed at 5 ¹/₂ years of age but required constant base therapy The other two died in infancy or early childhood, one from septicemia when 38 days old (20) the other at 2 years of age from a crisis of acidosis and dehydration during the course of a respiratory infection (18) Biochemically the disorder is characterized by the urinary excretion of large amounts of methylmalonic acid and by high plasma levels of this compound. It has been suggested that methylmalonic acid accumulates as the consequence of a deficiency of the mutase transforming L-methylmalonyl-coenzyme A to succinyl-coenzyme A (18) Isoleucine valine, threonine, methionine, thymine and fatty acids with an odd number of carbon atoms are precursors of methylmalonic acid.

The clinical features of methylmalonic acid mia very much resemble those of idiopathic hyperglycinemia (2), i.e. the ketotic type of hyperglycinemia (14). In this communication case of methylmalonic acidemia associated w th hyperglycinemia will be reported. Since one of the cases of methylmalonic acidemia which ha been reported also had an elevated plasma glycine level (20) there is evidence that hyperglycinemia with acidosis is the same disorder as methylmalonic acidemia. The possible connection of methylmalonic acidemia to congenital or infantile lactate acidosis (5) will also be discussed

CASE REPORT

The patient studied, E B., is girl bor September 1965 She is the second child of healthy unrelated parents. The older sibling is healthy boy

The mother had nausea and vomiting during almost the hole pregnancy Otherwise, pregnancy as uneventful. Delivery was without complications. Birth weight was 3730 g. During the neonatal period the baby vomited and lost much weight. Birth weight was not attained until one month of age She was breastfed for only 2 weeks. She then received cow s-milk formula of low protein content, providing her with about 2 g of protein per kg body ight and day Occasionally she vomited but seemed to thriv in other respects When 4 months of age the protein intak was markedly increased A few days fter the change of the feed the baby contracted mild upper respiratory tract infection with conjunctivitis During the course of that illness she had intense vomiting After about one eek she was admitted to the local hospital in state of moderate dehydration and severe metabolic acidosis

On admission the baby as lethargic and had marked muscular hypotonia Her weight was 5200 g. The liver as slightly enlarged Otherwise physical examination of the internal organs did not reveal any abnormalities There was massive ketonuria and low blood standard-bicarbonate level, 10 mEq per l In order to correct for the dehydration and acidosis, intravenous therapy ith fluid and electrolytes was immediately started. Initially there was rapid improvement but when the bicarbonate therapy was omitted after few days she relapsed with acidosis and signs of paralytic ileus. The serum potassium level

This investigation was supported in part by grant from AB Semper Fund of Nutritional Research

was low Intravenous therapy with fluid and electrolytes, including high amounts of sodium bicarbonate, had to be reinstituted. A diagnosis of infantile renal acidosis was suspected. Since, however the urine was found to be acid with pH of 5.0 and since there was no hyperchloremia such diagnosis was excluded.

When the baby had recovered from the two attacks she was maintained on sodium bicarbonate in a dose of 1 –18 mEq per day She then thrived and had normal blood standard-bicarbonate levels. When the therapy was discontinued after about 6 weeks she got an exacerbation with vomiting, lethargy severe muscular hypotonia and acidosis. It then became quite obvious that the metabolic acidosis was persistent and that the baby continuously required bicarbonate to be kept in normal acid-base balance. She also had tendency to develop hypokalemia which made it necessary to give her extra potassium. The baby was referred to Crown Princess Lovisa's Children Hospital for further work up.

Physical examination revealed 6-month-old girl with weight of 6410 g and length of 66.5 cm. The head circumference was 41.5 cm. She was responsive but gave the impression of being about 2 months retarded. She was quite unable to sit unsupported and had poor head control. She was hypotonic and had a poorly developed musculature. The anterior fontanel measured 1.0 by 1.5 cm. There were no skeletal abnormalities. Her throat, heart and lungs were normal. Blood pressure was 90/50 mm Hg. The liver was palpable 1 cm below the costal margin. Ophthalmological examination showed normal conditions. Repeated neurological examinations did not reveal any abnormalities except for the hypotonia.

Laboratory findings. On admission the hemoglobin was 12.1 g per 100 ml and the hematocrit value 37 per cent. The leucocyte count was 6700 per mm³ 17 per cent were neutrophils. The platelet number was 233,000 per mm³ The urine did not contain abnormal amounts of protein, glucose or ketone bodies, and no cells were seen in the sediment. The serum concentrations f sodium, potassium, chloride, calcium, and phosphate were normal, as were the blood levels f rea and glucose Standard-bicarbonate levels are given in Fig. 1 Studies f renal function revealed normal glomerular filtration rate as well as normal concentrating and acidifying capacities.

Roentgenograms of the long bones showed moderate osteoporosis but no signs of rickets. Electrocardiograms were normal The electroencephalogram which earlier had shown dysrythmic pattern was normal on repeated examinations. Electromyography showed normal pattern.

Course The baby continued to have exacerbations in association with dietary changes, with the contraction of acute infections or when the bicarbonate therapy was reduced. During the first 6 weeks after admission the feed was ordinary cow's-milk *formula. During that period* there were rather marked fluctuations of the weight but no net weight gain. When the cow's-milk formula was replaced by breast milk the baby started to gain weight, during 6-week-period there as in increase of 650 g. At discharge, at an age of 9 months, subcutaneous tissue was still scanty and she did not weigh more than 7130 g. However linear growth was normal, the length at 8 months of age as 70 cm. The growth of the head seemed to be somewhat arrested, the increase of the circumference was only 1 cm from 6 to 9 months of age.

The marked flaccidity and muscular hypotonia persisted during the period of observation. Thus, the baby was still quite unable to sit without support, and did not have full control of her head at 9 months of age. Psychological observations revealed psychomotor retardation. At 37 weeks the performance in the developmental test was about 30 weeks even when correction had been made for the muscular hypotonia. There were no seizures. During periods of hypokalemia the electro-cardiogram showed changes typical of such condition, otherwise it was normal. The neutropenia which was present on admission persisted until breast milk feeding was started. The number of neutrophils then increased from about 1100 per mm³ to between 2000 and 4000 per mm³ The hemoglobin level remained normal as did the platelet count.

When the baby was discharged to her local hospital she was on low-protein cow's-milk formula. T control *the metabolic acidosis 10 mEq of bicarbonate per day* was prescribed. The day after admission she became ill with an acute infection. After another day she developed severe acidosis and went into coma. The standard bicarbonate was as low as 7 mEq per l. The acute crisis as controlled by the intravenous administration f 36 mEq of bicarbonate during period of 2 hours. When the baby had recovered from this severe exacerbation she was prescribed bicarbonate in daily dose of 35 mEq On such therapy an ordinary but moderately protein restricted diet could be instituted at 11 months of age.

A marked general improvement of the psychomotor development was noticed at 14 months f age. The weight was, however only 8900 g. Although the girl was still markedly hypotonic she was able to sit without support. Hematologic findings were normal. Because of the good general condition the base therapy was discontinued. A few days later there was some vomiting lasting for day Without extra bicarbonate she had mild acidosis with standard bicarbonat varying between 18 and 20 mEq per l. Since, however she continued to be alert, had good appetite, did not vomit, and made satisfactory progress it was considered unnecessary to reinstitute the therapy She started to walk at 21 months of age.

The girl was last seen in her local hospital her 2 years and 3 months of age. She had no hypotonia and was able to run around. Her psychomotor development seemed to be normal for age. The weight was 14.7 kg and the height 88 cm. Although she had been without base therapy for more than year there had been no real exacerbations. A certain degree of protein restriction had been maintained. However periodically there had been moderate tachypnea. In the fasting state she had ketonuria. There was also persistent acidosis with standard *bicarbonate level of 15 mEq per l and blood pH of 7.31* (

METABOLIC STUDIES

Methods

Blood ketone bodies were determined as total acetone (24), blood lactate by an enzymatic

method (9), and blood pyruvate with a photometric microtechnique after formation of the 2,4-dinitrophenylhydrazone (12). The venous-plasma free amino-acid levels were determined with ion-exchange chromatography (11) by an earlier described modification (8), and the urinary amino acids either by the same method or by high voltage electrophoresis (10). Methylmalonic acid in serum and urine was determined according to Oberholzer *et al.* (18).

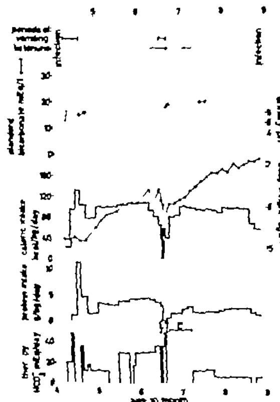


Fig. 1. Standard bicarbonate levels, urinary pH, caloric and protein intake and base therapy in the patient with methylmalonic acidemia. There were marked fluctuations of the standard-bicarbonate level. Very often the patient had marked acidosis. If the standard bicarbonate level is compared with the protein intake and the bicarbonate medication it is quite evident that the tendency to develop acidosis became much less when the protein intake was reduced. Citrate medication in a dose of 3.1 mmole per day (C in the figure) as well as treatment with tris (hydroxymethyl)-aminomethane (THAM) in one dose of 15 mEq (T in the figure) were effective in preventing acidosis. On the other hand, medication with lactate in a dose of 6 mEq 4 times in 24 hours (L in the figure) induced a crisis. The severe crises are initiated by trivial acute infections. Ketonuria was found only occasionally.

Table 1. *Fasting free amino acid levels in venous plasma on three different occasions*

The values are given in µmole/l. The normal levels are those given by Rompert (19). Serine and threonine were not determined.

Amino acid	Age of the patient: 7 months	8 months	1 1/2 years	Normal levels, 9 months to 2 years: Mean	Range
Alanine	455	228	282	219	99–313
Glycine	804	250	231	170	56–308
Valine	96	1 1	261	127	57–262
Lysine		115		87	45–144
Leucine	13	69	177	75	45–155
Taurine	53	62	284	49	19–91
Arginine		88		31	11–65
Histidine		76		64	24–112
Isoleucine	5	32	91	44	26–94
Phenylalanine	11	51	75	40	23–69
Glutamic acid	218	183	135	135	44–290
Tyrosine	25	56	48	45	11–122
Methionine	7	6	18	21	3–29
Citrulline	T	9	13		
-NH_2-BU	Tr	9	5	5	0–17
Aspartic acid	12	8	7	2	0–9
Cystine	<3	8		4	0–40

Results

On admission a diagnosis of hyperglycinemia with acidosis (7) or infantile lactate acidosis (5) was suspected.

Acid-base balance. The blood standard-bicarbonate level, pH of the urine, base therapy and dietary intake of calories and protein are given in Fig. 1. There was a chronic metabolic acidosis which had to be corrected by bicarbonate, the dose which was required varied with the protein intake. On a low intake less bicarbonate was required than when it was high. The pH of the urine varied with regard to the protein intake. When it was high the pH was very low varying between 5.0 and 4.5 although extra bicarbonate was given. During the course of acute infections large amounts of bicarbonate were necessary. Acidosis was not prevented by sodium lactate. On the contrary the administration of lactate initiated an acidotic crisis. THAM and potassium citrate proved, however, to be effective.

Free amino acids in urine and plasma. When the baby was 7 months old paper chromatography of free amino acids in the urine showed a marked hyperglycinuria. High voltage electrophoresis re-

Table _ *Blood levels of ketone bodies, lactate and pyruvate*

The determinations were made when the patient was 7 months of age.

	Number of determinations	Range (mmole/l)	Mean (mmole/l)
Ketone bodies	4	0.19–0.40	
Lactate	3	2.3 5.6	3.9
Pyruvate	3	0.16–0.50	0.21

vealed a urinary excretion of about 400 mg of glycine in 24 hours. Glycine was the only amino acid to be excreted in excess. At $2\,^{8}/_{13}$ years of age the urinary excretion of glycine was less than at the first determination. By the column chromatographic method it was found that the 24-hours excretion of glycine had dropped to 170 mg. At the same time the daily excretion of alanine was _5 mg.

When the urinary excretion of glycine was very high there was also a marked hyperglycinemia (Table 1). When the plasma amino acid levels were determined after the baby had received a protein restricted feed for about 3 weeks the glycine level was almost normalized and remained so at the time of the last determination at _ $_{13}$ years of age When free plasma amino acids were estimated for the first time, the alanine level was found to be elevated in addition to that of glycine, whereas the levels of leucine, isoleucine and valine were very low The ery low concentrations of the branched-chain amino acids might be explained by the fact that the sample was taken just a few days after an acute crisis, when the baby most likely was in an anabolic phase after a state of acute protein starvation. In the last determination of free plasma amino acids the levels of leucine, isoleucine, and valine were found to be in the upper normal range Also, the taurine level was elevated.

Blood levels of ketone bodies lactate and pyruvate The results are given in Table 2. As can be seen the blood level of ketone bodies was normal. However during crises ketone bodies appeared in the urine (Fig. 1) The blood lactate level was found to be elevated The ratio of lactate to pyruvate varied between 5 and 70.

Methylmalonic acid in plasma and urine The plasma level and urinary excretion of this compound was determined in December 1967 when the patient was $2\,^{8}/_{13}$ years old. As can be seen from Table 3 large amounts of methylmalonic acid were excreted in the urine. The plasma level was also found to be abnormally high.

Table 3 *Plasma level and urinary excretion of methylmalonic acid*

Plasma level	2.4 mg per 100 ml
Urinary excretion	1340 mg per 24 hours

COMMENTS

The cardinal clinical feature in our patient was chronic metabolic acidosis which partly could be controlled by restriction of the protein intake. Periodically exacerbations with vomiting, dehydration, lethargy coma, and even peripheral circulatory failure appeared. Trivial acute infections, overloading with protein and the administration of lactate were found to induce life-threatening crises. As long as the baby remained severely acidotic there was marked muscular hypotonia and retarded development. The weight gain was slow Initially there was neutropenia, but the neutrophil count was normalized after the protein intake had been restricted. During infancy the baby required continuous therapy with bicarbonate to control the acidosis. At an age of about 18 months there was a remarkable improvement. She then started to gain weight and the psychomotor development became accelerated. The base therapy was then omitted without any consequences but a mild metabolic acidosis. A restricted protein intake has been maintained, however

During the acute episodes our patient excreted ketone bodies in the urine. It was also possible to induce ketonuria by loading the baby with high amounts of protein. However accumulation of ketone bodies could not account for the metabolic acidosis since it persisted even when there was no ketonuria, and the level of blood ketone bodies was normal. In search for other organic acids contributing to the acidosis, it was found that the blood lactate level was elevated It was, however obvious that hyperlactatemia could not be the sole reason for the metabolic acidosis since there was no correlation between the variations of the blood lactate concentration and the standard-bicarbonate level. First by the demonstration of an increased

Table 4. Summary of multiple biochemical abnormalities in methylmalonic acidemia, hyperglycinemia with acidosis, and infantile lactic acidosis

	Methylmalonic acidemia			Hyperglycinemia with ketosis	Infantile lactic acidosis
Reference	This report	(18)	(20)	(2, 17, 23)	(4, 5, 6, 13)
Number of cases	1	1	1	3	6
Metabolic acidosis	+	+	+	+	
Ketosis	Transient	Transient	Transient	Transient	
Hyperlactatemia	+	Unknown	Unknown	Unknown	
Hyperglycinemia	Transient	Unknown	+	Transient	Unknown
Hyperglycinuria	Transient	Unknown	+	Transient	Unknown
High blood levels of branched-chained amino acids	Transient	Unknown	Unknown	Transient	Unknown
Accumulation of methylmalonate	+	+	+	Unknown	Unknown
Intolerance to high protein load	+	+	+		( )

plasma level and a large urinary excretion of methylmalonic acid was it possible to explain the pathogenesis of the metabolic acidosis.

The finding in our patient of a high urinary excretion of methylmalonic acid is in accordance with a diagnosis of methylmalonic acidemia (20) or aciduria (18). The clinical features were also the same as those in the only reported surviving patient with that disorder. On the other hand, the symptoms in our patient were typical of the ketotic type of hyperglycinemia (2, 14). In fact, such a diagnosis was also made when the occurrence of hyperglycinemia and hyperglycinuria was established. However, on the basis of some of the clinical and biochemical features a diagnosis of infantile lactic acidosis (5) may also be considered. The blood lactate level was found to be elevated to the same extent as has been found in that condition (4, 6). Furthermore, the intolerance to lactate, as manifested by the development of an episode leading to coma following the administration of lactate, seems to imply an abnormality in lactate metabolism.

Due to the great similarities between hyperglycinemia with ketosis, infantile lactic acidosis, and methylmalonic acidemia it seems relevant to consider whether they in reality constitute different biochemical manifestations of the same disorder. Stokke *et al.* (20) in their report on a case of methylmalonic acidemia have already made the suggestion that hyperglycinemia and methylmalonic acidemia are the same disorder. For comparison, the biochemical abnormalities of the three conditions are listed in Table 4. Since many data are lacking regarding the 6 reported cases of so-called infantile lactic acidosis (4, 5, 6, 13), it is impossible to establish whether this syndrome has any relation to methylmalonic acidemia. The only biochemical evidence for such a hypothesis is the presence of hyperlactatemia in our patient with methylmalonic acidemia. On the other hand, no data are available about the blood lactate level in the two thoroughly investigated cases of the ketotic type of hyperglycinemia which have been reported (2, 23). The assumption that methylmalonic acidemia and the ketotic type of hyperglycinemia are the same disease seems, however, to be well documented. In our patient with methylmalonic acidemia there was only mild and transient ketosis whereas ketosis is reported to be severe in the ketotic type of hyperglycinemia (14). This discrepancy does not exclude that the disorders are the same since the tendency to develop ketosis may be subject to individual variations. This suggestion is supported by the findings in a 20-months-old boy with methylmalonic acidemia who is now under investigation in our department. In this patient the blood level of ketone bodies was enormously elevated, i.e. 14.0 mmole per l when the daily urinary excretion of methylmalonic acid was 3650 mg and the plasma level 23 mg per 100 ml. It would be interesting to know the urinary excretion and plasma level of methylmalonic acid in other patients with the ketotic

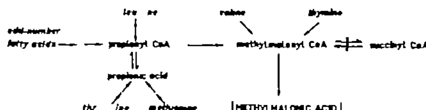


Fig 2. Pathways of the formation of methylmalonic acid. The probable metabolic block in methylmalonic acidemia is indicated.

type of hyperglycinemia. This might be possible since one of 9 cases which have been reported with the diagnosis of ketotic hyperglycinemia was *still alive and in good condition in 1966* at an age of 3 years (14)

The pathogenesis of methylmalonic acidemia is suggested to be a partial metabolic block between L-methylmalonyl-coenzyme A and succinyl-coenzyme A due to a deficiency of methylmalonyl coenzyme A mutase (18) an enzyme requiring cobamide coenzyme (7) cf. Fig. 2. If hyperglycinemia with ketosis and methylmalonic acidemia are the same disorder the biochemical abnormalities typical of both conditions have to be explained on the basis of the same metabolic defect.

In the ketotic type of hyperglycinemia loading with the 5 amino acids leucine, isoleucine, valine, threonine, and methionine has been found to induce acute episodes (1 15), whereas 11 other amino acids were found to be beneficial by reducing the toxicity of the first mentioned amino acids. *Glycine was found to be absolutely non*toxic (1) As is shown in Fig. _ all those amino acids inducing symptoms in hyperglycinemia with ketosis are except for leucine, precursors of methylmalonyl-coenzyme A. The toxic effect of leucine might be explained by its ketogenic property Tolerance tests have shown that the removal of leucine from the blood is slow in hyperglycinemia with ketosis (15). Also the other two branched-chain amino acids may accumulate in the blood in that condition (15) The demonstration in our patient of plasma levels of leucine, isoleucine, and valine in the upper normal range is in agreement with these findings. The results might be evidence of a slow degradation of leucine, isoleucine, and valine. A tentative explanation for such an abnormality would be that an intracellular accumulation of methylmalonyl-coenzyme A reduces the amount of coenzyme A being available for the metabolism of the branched-chained amino acids. The intense ketosis, as seen in hyperglycinemia with ketosis and in our second patient with a confirmed diagnosis of methylmalonic acidemia, might also originate from a relative deficiency of coenzyme A.

Accumulation of glycine and attacks of acidosis have been considered to be the diagnostic criteria of hyperglycinemia with ketosis. It has been suggested that the high plasma glycine level and the doubled size of the glycine pool are due to an inefficient conversion of glycine to serine (16). However in view of the fact that the plasma level of serine has been found to be slightly elevated in hyperglycinemia with ketosis (15) a slow conversion of glycine to serine might be a secondary phenomenon without any significance for the development of hyperglycinemia. The fact that the major pathway for serine metabolism probably is conversion to glycine (3) is in favor of such a *statement. If other causes of hyperglycinemia have* to be considered, it may be pointed out that a new pathway which could account for a rapid oxidation of glycine has been demonstrated in guinea pig liver (1) Glycine condenses in the mitochondria with acetyl-coenzyme A to form α-amino-β-ketobutyric acid, which decarboxylates to aminoacetone. In methylmalonic acidemia this pathway may be inefficient because of lack of coenzyme A.

The hyperlactatemia and intolerance to lactate as seen in our patient might be explained by the fact that methylmalonyl-coenzyme A is a very effective competitive inhibitor for the carboxylation of pyruvate to oxalacetate the first step in gluconeogenesis from pyruvate (22).

In those patients reported as cases of the ketotic type of hyperglycinemia as well as in those

found to accumulate large quantities of methylmalonate trivial infections have been of extreme seriousness. This is most likely due to the fact that acute infections are associated with some tissue breakdown and negative nitrogen balance. Under the condition of a block in the degradation of several amino acids and of thymine, acute infection easily may cause severe metabolic derangement

SUMMARY

The clinical and biochemical features of a case of methylmalonic acidemia, a newly described inborn error of metabolism, are reported.

The cardinal feature was chronic metabolic acidosis which was controlled by restriction of the protein intake. Periodically exacerbations with vomiting, dehydration, lethargy coma, and even peripheral circulatory failure have appeared. The crisis have been induced by trivial acute infections and by overloading with protein. Mild symptoms appeared in the neonatal period, crises after an age of 4 months. When the protein intake was high, there was marked muscular hypotonia, developmental retardation, and neutropenia. When acidosis was controlled by protein restriction and treatment with bicarbonate the baby made marked progress. At 2 ³/ years of age the development was normal.

The patient excreted large amounts of methylmalonic acid in the urine and the blood level was elevated. Other biochemical abnormalities were hyperglycinemia, hyperglycinuria, and hyperlactatemia.

The hypothesis that the basic metabolic defect i methylmalonic acidemia and the ketotic type of hyperglycinemia is the same is discussed, as are the similarities between methylmalonic acidemia and infantile lactic acidosis.

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THE MECHANISM OF DIARRHOEA IN CONGENITAL DISACCHARIDE MALABSORPTION

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Recent research had added considerably to our knowledge of the hydrolysis and absorption of disaccharides and of the clinical conditions associated with their inadequate splitting (e.g. 22) Our insight into the pathophysiologic action of unabsorbable disaccharides in the intestines, however is rather deficient. On the basis of the high lactic acid content of faeces it has been suggested that bacteria-induced fermentation of disaccharides may have a central role in the aetiology of the diarrhoea caused by unabsorbed disaccharides (27). On the other hand, there are roentgenologic observations of contrast medium which contains unabsorbable disaccharide being diluted in the small intestine and transit through the small intestine being shortened (18) The recent method of intestinal intubation has also brought about evidence suggesting that lactose causes movement of water into the lumen of the small intestine in patients with lactase deficiency (11)

The object of the present study is to clarify the absorption of sucrose and lactose in three children with congenital disaccharide malabsorption, and the action of these disaccharides on the movements of water and electrolytes in the gastrointestinal tract to obtain information on the mechanism of the diarrhoea associated with disaccharide malabsorption.

PATIENTS

Two infant siblings (M. J and K. J) with CLM and one infant (H. K.) with CSIM were studied. The clinical data and intestinal biopsy findings have been presented elsewhere (15 17). Patients M. J and K. J had been kept on lactose-free diet for nine and two months, respectively prior to the investigations and patient H. K. on sucrose free diet for three weeks. Patient M J was studied using infusion method both before and after three-week period of milk containing diet.

This study was supported by the Paulo Foundation and the Foundation for Pediatric Research in Finland Following abbreviations are used. CLM congenital lactose malabsorption, CSIM – congenital sucrose-isomaltose malabsorption PEO – polyethylene glycol, ECF – extracellular fluid

METHODS

Intestinal intubation technique

Data on the intestinal intubations performed and the tubes used are given in Table 1 and Fig. 1. The 2-channel tubes had the opening of each channel at different distance to permit simultaneous sampling from two different levels. A mercury filled rubber bag (Breuninger, Stuttgart, West-Germany) was used as weight (Fig 1). The tube was introduced transnasally after local anesthesia with Xylocain® gel. Its passage was followed by fluoroscopy The tip reached the distal ileum in week on an average (Table 1).

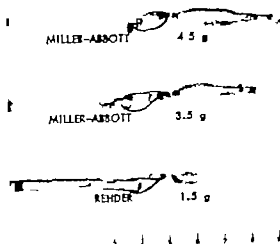


Fig 1 The tubes with mercury filled rubber bags used in the intestinal intubations.

Table 1. *Data on the intestinal intubations performed*

Patient	Diagnosis	Weight of patient (kg)	Age at intubation (months)	Tube	Time in which distal ileum was reached (days)	Total duration of intubation (days)
Test fluid as meal						
M. J	CLM	7 7	10	Rehder[a]	10	11
K. J	CLM	4 0	2	Miller Abbott[b]	8	11
H. K.	CSIM	7 7	7	Miller-Abbott	4	7
Test fluid as constant infusion						
M J	CLM	10 4	19	Rehder	5	12
		10.4	20	Rehder	8	10
K. J	CLM	4 4	3	Miller Abbott	2	6

The tubes were constructed from
[a] Rehder Jejunalsonde nach Rehder Rtg.-posit., Ch 9 Ø 3 mm, Willy Rüsch KG N 4547
[b] Miller–Abbott, bilumen Miller–Abbott, Rtg.-posit., Ch 12, Ø 4 mm, Willy Rüsch KG No 2050 b.

Experimental design

T different techniques were employed. test meal and infusion. Test meals both of sucrose- and of lactose containing solution (see Table 2) were given to all three patients. When the tube was in proper position, the test solution, 200 ml for patients M.J and H.K. and 140 ml for patient K.J was fed from bottle, and sampling of the intestinal contents at 5–10 min intervals was begun immediately The samples were taken from proximal jejunum, distal jejunum and distal ileum. With 2-channel tube (Table 1) it was possible to take samples from tw different levels at one investigation. After test meal containing absorbable disaccharide were samples obtained in the distal ileum of only one patient (M.J sucrose ng solution). Stools were collected for a maximum of 4 hr

The infusion technique was used only in the patients with CLM. Patient M.J was studied both before and after the three-week period f milk-containing diet. When the tube had reached the distal ileum, infusion of test solution (Table 2) at constant speed (1 ml/min) through feeding tube into the stomach was started, using a roller type pump. T obtain steady state, this was continued for minimum of 12 hr (sucrose solution) or 6 hr (lactose solution) prior to collection of samples. Collection of stools was then begun, and for sampling of the intestinal contents at different levels, the tube is stepwise withdrawn. Two successive samples were drawn from each level first allowing 10–15 min equilibration.

Every stool passed and the intestinal-fluid samples were placed immediately at −20°C and kept there until analysis.

Table 2. *Composition of the test fluids*

	Test meals	Constant infusions[a]
Disaccharide, lactose or sucrose	150 mmoles/l	150 mmoles/l
Polyethylene glycol 4000 (PEG)	5 0 g/l	5.0 g/l
N	76 mEq/l	26 mEq/l
K	5.4 mEq/l	7.6 mEq/l
Ca^{++}	3.4 mEq/l	—
Cl^-	82 mEq/l	34 mEq/l
HCO_3^-	2.4 mEq/l	—
Glucose	—	50 mmoles/l
l-Alanine	—	50 mmoles/l
Palmitic acid	—	5 mmoles/l
Lecithine (soy-bean)	—	5 g/l
Total osmolality	320 mOsm/kg	384–425 mOsm/kg (mean 408)

The test fluid was prepared as described elsewhere (14).

Chemical analysis

For determination of PEG and sugars in the intestinal fluids and the stools (the latter first diluted 1 10 with water and homogenized) precipitation with $Zn(OH)_2$ was performed. PEG was determined with modification (13) f the method of Hyden (9).

Glucose was determined with glucose oxidase method using Tris-glucose xidase reagent (2). Sucrose was determined as glucose after complete hydrolysis with invertase in 0.1 M acetat buffer at pH 4.6 (1) and lactose as glucose after deionization f the filtrate with the ion exchange resins Amberlite IR 120 and Amberlite IRA-400 (OH form, freshly prepared), and complete hydrolysis with lactase (Mann Research Laboratories) in 0.05 *M* sodium maleate buffer at pH 6.8

The -lactate content was determined with specific enzymic method (24) from intestinal fluids after precipitation with per chloric acid or $Zn(OH)_2$, and from stools after precipitation with $FeCl_3$ and $Ca(OH)_2$ (27). Sodium and potassium were determined with flame photometer ("Eppendorf") and chloride with coulometric titration (EEL Chloride Meter)

Osmolality was measured with freezing point osmometer (Advanced Instruments) after centrifugation of the samples. The α-amino nitrogen content of the intestinal

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Constructed by M T Ahola.

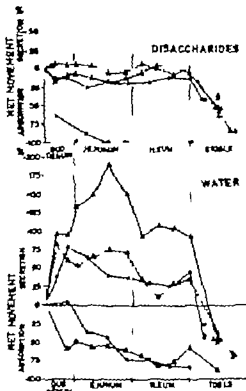


Fig. 3. Net movement of disaccharides and water in the gastrointestinal tract of the patients with CLM, when the test fluid containing either lactose (black symbols) or sucrose (open symbols) was infused into the stomach. Symbols: △, Patient MJ; ○, patient KJ. Broken line indicates investigation in patient MJ after the 3-week ingestion of a lactose-containing diet.

Fig. 2 a–c. Net movement of disaccharides, water and sodium, after the test meal containing either lactose in the patients with CLM and sucrose in the patient with CSIM (black symbols) or sucrose in the patients with CLM and lactose in the patient with CSIM (open symbols).

fluid was determined by the ninhydrin method (21), using alanine as standard. The amino acid pattern was studied with high voltage electrophoresis (28).

Faecal sugars were identified with thin layer chromatography (16). When the stools were first diluted 1:5 with water, homogenized and centrifugated.

Calculations

The degree of absorption of water at the different levels of the intestinal tract was calculated from the following formula: net movement % = 100 [(P P) − 1] and that of solutes from the following formula: net movement % = 100 [(S P) (P S) − 1], in which S is the concentration of the solute and P the concentration of PEG in the sample, and S_0 the concentration of the solute and P the concentration of PEG in the test fluid. A negative sign indicates net movement out of and a positive sign into the lumen. In the test meals the sample (or the samples using mean values), in which the PEG content was highest was selected to represent each level.

When the ion concentrations were converted to osmolality units available tables were used (1). In calculating how much of the lactose or sucrose was found in

Table 3 *The concentrations[a] of disaccharide and electrolytes in the intestinal contents at different levels after test fluid meals in an infant with congenital sucrose-isomaltose malabsorption and two infants with congenital lactose malabsorption*

		Nonabsorbable disaccharide in test fluid[b]			Absorbable disaccharide in test fluid[c]		
	Test fluid	Proximal jejunum	Distal jejunum	Ileum	Proximal jejunum	Distal jejunum	Ileum
Lactose or sucrose, mmoles/l	150	105 (100–109)	106 (94–114)	96 (87–113)	56 (44–69)	24 (9–53)	0
N mEq/l	76	89 (88–89)	89 (84–92)	91 (86–99)	90 (82–98)	98 (90–110)	147
K mEq/l	5 4	5 4 (4.9–5.8)	4.5 (4.2 5 0)	4 4 (3.8–4.8)	7.9 (6.5–8.3)	8.5 (7 6–9.3)	5 1
Cl⁻ mEq/l	82	92	93 (90–96)	94 (89–98)	96 (88–104)	105 (92–119)	124
Osmolality calculated, mOsm/kg	320	293 (288–297)	293 (286–303)	289 (269–302)	261 (257–265)	243 (215–257)	308

Mean and range.
[b] Sucrose in patients with sucrose-isomaltose malabsorption and lactose in patients with lactose malabsorption.
Lactose in patients with sucrose-isomaltose malabsorption and sucrose in patients with lactose malabsorption

the stools as monosaccharides or lactate (Fig. 4), it was assumed that one molecule of glucose corresponds to one molecule of disaccharide and that two molecules of lactic acid are formed via glucose from one disaccharide molecule (4).

RESULTS

The results of the test meals are shown in Figs. 2 1 4 and in Table 3 and those of the infusions Figs. 3 4 and 5 and in Table 4

Splitting and absorption of disaccharides

It appears from Figs. 2 *a* and 3 that the absorbable disaccharide (sucrose in CLM and lactose in CSIM) was completely absorbed already in the proximal part of the small intestine. In contrast, the unabsorbable disaccharide (lactose in CLM and sucrose in CSIM) hardly disappeared at all from the small intestinal lumen (Figs. 2 *a* and 3): an average of only 8% of the disaccharide administered had disappeared. However the disaccharide which had failed to be absorbed in the small intestine was seen partly to disappear in the colon. Fig. 4 shows that in both types of study the CLM patients had 50–80% of the administered lactose unsplit in the stools, 1.5–16% was excreted as monosaccharides and a very small part, 0 4–0.9% as lactate. In the CSIM-patient only 15% of the sucrose given came through unsplit, 27% was established as monosaccharides and 0 7% as lactate.

Movement of water and electrolytes

Alongside the normally digested disaccharide there was absorption of water and the volume of the intestinal contents decreased continuously on moving in the distal direction (Figs. 2 *b* and 3)

In contrast, secretion of water and Na -salts into the intestinal lumen occurred in the presence of lactose in the CLM-patients and in the presence of sucrose in the CSIM-patient (Figs. 2 *b* 2 *c* and 3) and resulting in 1.5–2.5 fold dilution of the test fluid in the duodenum and proximal jejunum. The osmolality of the intestinal contents, however kept in the range of 276–296 mOsm/kg, mean

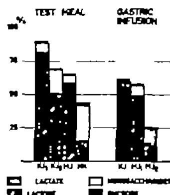


Fig. 4 The recovery of lactose in the CLM-patients and sucrose in the CSIM-patient in the stools intact, and as monosaccharides and lactate in per cent of the total amount of the disaccharide administered. MJ indicates investigation in patient MJ after the 3-week ingestion of lactose-containing diet.

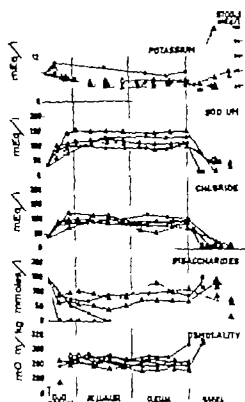


Fig. 5. The osmolality and concentrations of electrolytes and disaccharides in the intestinal contents at different levels of the CLM patients when the test fluid containing either lactose (black symbols), or sucrose (open symbols) was infused into the stomach. Symbols: Δ, Patient MJ. O patient KJ. Broken line indicates investigation in patient MJ after the 3-week ingestion of lactose-containing diet.

287 mOsm/kg (Fig. 5) of which the unabsorbed disaccharide accounted for 25–30%. Also when sucrose was infused, the osmolality of the intestinal contents of the CLM-patients was the same, 280–310 mOsm/kg, mean 288 mOsm/kg (Fig. 5).

In infusions of the CLM-patients with the lactose fluid absorption of water took place already in the distal jejunum and in the ileum (Fig. 3); this was not seen after the test meal. The content of water in the intestinal fluids was 28–?7% smaller in the ileum than in the jejunum. However the osmolality of the intestinal contents remained unchanged and there was no disappearance of lactose. The lactose concentration increased correspondingly and the Na-concentration decreased (Fig. 5).

Extensive absorption of water and N -salts occurred in the colon (Figs. 2 b and 3). With both techniques, the content of water in the stools was smaller than in the test fluid and the faecal Na concentration was distinctly smaller than in the ileal fluids (Fig. 5).

The average K-level of the contents of the small intestine was 4.4–6.6 mEq/l in studies with fluid containing unabsorbable disaccharide with both techniques (Fig. 5 and Table 3). When the absorbable disaccharide was administered, the average K-concentration of the content of the small intestine was 5.0–10.5 mEq/l (Fig. 5 and Table 3). After infusion of lactose in CLM-patients, the K-concentration of the stools was 16–30 mEq/l (Fig. 5). When these concentrations were related to the movement of water it appeared that slight secretion of potassium had occurred in the colon.

The Cl-concentration of the small intestinal contents was distinctly lower than the sum of Na+ and K (Fig. 5 and Table 3). The lactate concentration was too small (Table 4) to explain this difference. No significant differences established in the lactat concentration in the small intestine when the CLM-patients were given infusions containing sucrose or lactose.

Table 4. *Lactate concentrations[a] in the intestinal contents at different levels in 2 infants with congenital lactose malabsorption on infusion of test fluid into the stomach*

	mEq/l Duodenum	Jejunum	Ileum	Stools
Sucrose containing test fluid	1.0 (0.9–1.0)	1.1 (0.5–2.2)	1.2 (0.3–2.3)	—
Lactose containing test fluid	0.4 (0.3–0.5)	0.5 (0.3–0.8)	0.5 (0.2–0.9)	3.3 (1.8–5.2)
Lactose containing test fluid, after lactose containing diet (patient M. J.)	0.3 (0.3–0.5)	0.2 (0.1–0.5)	0.2 (0.1–0.3)	57 (38–76)

[a] Mean and range.

Absorption of glucose and alanine

The glucose and l-alanine in the infusions disappeared almost completely from the lumen in the proximal part of the intestine. Distally from mid-jejunum the contents of the small intestine had a nearly constant amount of ninhydrin-positive sub-

stances which high-voltage electrophoresis demonstrated to be mostly amino acids. The amino acid pattern was rather invariable.

Effect of lactose-containing diet on the findings in a CLM-patient

As shown in Figs. 3 and 5 the 3-week period during which patient M. J was on a lactose-containing diet had little effect on the phenomena measured in his small intestine. In the small intestine no significant disappearance of lactose from the lumen was observed and the lactate concentrations were actually a little lower (mean 0.2 mEq/l) than in the first investigation (mean 0.4 mEq/l) By contrast, the lactose content of the stools was distinctly smaller than earlier only an average of 12% of the lactose administered was found unsplit in the stools (Fig. 4). The average lactate concentration of the stools increased from 2.3 mEq/l to 57 mEq/l (Table 4) However the volume of the stools was now only 32% of the volume of the infusion solution having been 56% in the earlier investigation (Fig. 3)

DISCUSSION

This investigation shows that normally hydrolysed disaccharides disappear from the intestinal lumen in the proximal part of the small intestine, whereas there is hardly any disappearance of unhydrolysed disaccharides.

The unhydrolysed disaccharides caused considerable net movement of electrolytes, especially NaCl, and water into the lumen right in the proximal part of the gastrointestinal tract so that the test solutions were diluted 1 1/2– 2 1/2-fold and their Na and Cl⁻-concentrations approached corresponding concentrations in ECF The intestinal contents became or remained iso-osmotic with ECF As the contents of the small intestine contained throughout unabsorbed disaccharide which in average made up of a third of the osmotic activity of the intestinal contents, the Na and Cl⁻ concentrations did not attain the level of the ECF concentrations.

Substances like mannitol which are unabsorbed (7 21) or such as xylose which are only slowly absorbed (5) are known when infused into the intestine to cause movement of water into the lumen. Similarly lactose, when infused into adults with (acquired) lactase deficiency as an iso-osmotic solution causes movement of water into the lumen (11) Kern & Struthers (11) noted that the electrolyte free lactose solution trebled in volume in a 100 cm long segment of small intestine and that the intestinal contents throughout were iso-osmotic with plasma. This means that unabsorbed lactose finally contributed about a third of the osmotic activity of the intestinal contents. This phenomenon is here demonstrated also in infants with congenital disaccharide malabsorption.

It is apparent that the small intestine tends to render and keep its contents iso-osmotic with ECF (5 6 10 20 26). In addition, it tends to equilibrate the Na -concentration with the ECF (5). How quickly these states of equilibrium can be achieved depends on the amount of other solutes in the intestinal contents and their rate of absorption. If the lumen contains completely unabsorbable solutes, iso-osmolality with ECF is achieved fairly rapidly but the intraluminal Na -concentration does not reach that of ECF despite the increase in the intraluminal volume. In disaccharide malabsorption, the endeavour to achieve states of equilibrium leads to considerable binding of water and electrolytes in the small intestine, i.e. a considerable increase in the intraintestinal volume.

The present results suggest that ileum better than jejunum is able to maintain a Na^+ gradient with ECF In the infusion studies of the CLM patients the volume and the concentration of Na^+ were smaller in the ileum than in the jejunum and, the mean lactose concentrations correspondingly higher in the ileum than in the jejunum. This concurs well with the observation of Fordtran and his co-workers (7) that the upper intestine has a much higher permeability to the bulk flow of water than does the lower small intestine.

There is no evidence that fermentation of the unabsorbed disaccharides occurred in the ileum for no disapparance of disaccharides was observed and the lactate concentration of the contents was unchanged and very low

In the colon there was disappearance of the disaccharides manifest in both the CLM and CSIM-patients. A part of the disaccharides was split to monosaccharides and only a very small proportion to lactate The disappearance of sucrose was greater in the CSIM-patient than that of lactose in the CLM cases. This may be due to greater ability of the bacterial flora in the colon of the former to split sucrose than that of the latter to digest lactose However also the bacterial

flora of CLM-patients may become adapted to better split lactose. In the investigation of patient M. J after 3 weeks on lactose-containing diet no more than 12% of the lactose administered was found in the stools whereas the mean lactate concentration, 57 mEq/l was considerably higher than in the investigation before this diet (2.3 mEq/l) This increase in the formation of lactic acid was thus confined to colon and had no effect on the phenomena measured in the small intestine. On the other hand, it was surprising that the faecal volume was smaller average 32% of the volume of the infusion solution, than before the lactose-containing diet when it averaged 56% It seems that the unabsorbed disaccharide retained water effectively as far as the colon. In the colon the disaccharide was split into monosaccharides and further to lactate, these substances were absorbed from the colon and the faecal volume decreased correspondingly At least glucose is previously known to be absorbed from the colon (8 25)

Several differing theories of the mechanism of diarrhoea in sugar malabsorption have been put forward in the course of the years. Lindquist & Meeuwisse (19) on the basis of the high sugar concentration of the stools in their patient with glucose-galactose malabsorption suggested the osmotic effect of sugars in the intestines as the fundamental cause of diarrhoea. On the other hand, Weijers and his co-workers noted that the stools of patients with disaccharide malabsorption contain great amounts of volatile organic acids, especially acetic acid and lactic acid, as the result of bacterial fermentation. They assumed that "these acids, and possibly also other metabolites of the bacterial flora, irritate the intestine, which reacts to this with increased peristalsis, excretion of fluid and mucus formation, due to which the absorption is disturbed, with subsequent diarrhoea (27). The role of volatile organic acids as the causative agent in diarrhoea is a central factor also in an other hypothesis, based on roentgenologic findings, that the volatile fatty acids produced by fermentation in the colon have a pronounced osmotic effect resulting in profuse secretion of water to the colon and ensuing diarrhoea (12). The observation by Laws & Neale conflicts with this hypothesis (18) When they gave patients with lactose and sucrose malabsorption lactose and sucrose, respectively together with the contrast medium, they noted that the contrast medium thinned out in the small intestine and that passage through the small intestine was shortened.

I propose on the basis of the present findings the following pathogenesis of the disaccharide diarrhoea. Unabsorbed disaccharide causes in the proximal part of the small intestine pronounced movement of water and electrolytes into the lumen until the contents are in osmotic equilibrium with ECF and the unabsorbed disaccharide only contributes roughly a third of the osmotic activity The question whether increased volume in turn stimulates the passage through the intestine cannot be answered from the results of present investigation.

In the colon part of the disaccharide disappears through bacterial fermentation, and there is absorption of water and electrolytes even in relative excess of that disappearance. Yet enough fluid remains to give diarrhoeal stools.

The diarrhoea is thus due to the osmotic activity of the disaccharide in the intestine together with the organism's tendency to Na^+-equilibrium between the intraluminal and extracellular fluids. There is no evidence to suggest that the bacterial fermentation of the disaccharide in the colon had an etiologic role in the diarrhoea, e.g. through inhibiting absorption.

SUMMARY

Intestinal malabsorption of lactose and sucrose, and the effect of these disaccharides on the movements of water and electrolytes were studied in two siblings with congenital lactose malabsorption (CLM) and one infant with congenital sucrose-isomaltose malabsorption (CSIM) using the intestinal intubation technique.

Sucrose in the CLM-patients and lactose in the CSIM-patient were absorbed already in the proximal part of the small intestine, whereas hardly any lactose in CLM and sucrose in CSIM disappeared from the small intestinal lumen. The unabsorbed disaccharides caused considerable movement of water and electrolytes into the intestinal lumen test fluid being diluted to 1 /$_{3-}$1/ times in the duodenum and proximal jejunum. The contents of the small intestine were throughout isoosmotic with extracellular fluid and the unabsorbed disaccharides represented 25–30% of the osmotic activity

In the colon, the unabsorbed disaccharides dis-

appeared in varying degree, they were split into monosaccharides and converted to lactate. Water Na^{+} and Cl^{-} were absorbed in the colon even in relative excess to the disappearance of the disac charide and its splitting products, and in all cases the faecal volume was smaller than the volume of the test fluid In a CLM-patient a 3-week period on lactose-containing diet had no effect on the phenomena demonstrated in the small intestine, but disappearance of lactose and formation of lactate was greater in the colon, and amount of water less in the stools than before.

It is suggested that the retention of water in the intestinal lumen through the osmotic activity of the unabsorbed disaccharide, together with the organism s tendency to Na -equilibrium between the luminal and extracellular fluid is the most important mechanism of diarrhoea in disaccharide malabsorption.

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DIURNAL VARIATION OF SERUM IRON IN INFANTS AND CHILDREN

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The circadian variation of serum iron in healthy adults is related to the sleeping cycle. Normal individuals have maximum serum iron levels in the morning, with the lowest levels about 12 hours later while the reverse is true in night workers (6, 15). A similar pattern has been observed in the variation of a wide variety of physiologic functions and levels of chemical substances in the blood. This diurnal variation is not present at birth in most infants. Rutenfranz (13) has noted the variable age of attainment of circadian rhythms of body temperature, heart rate, sleep pattern, and urinary cation excretion. An adult pattern of variation of plasma 17 hydroxycorticosteroids does not appear until one to three years of age (3). The present study evaluates the age of onset of diurnal variation of serum iron in a pediatric population.

MATERIAL AND METHODS

Twenty-four infants and children without anemia were selected from the hospital nurseries and wards. None of the children were severely ill or infected at the time of study. The diet, physical activity and sleep pattern of each child were carefully observed. Twelve infants varied in age from 2 weeks to 21 months. None of these infants were younger than 3 months old. The remaining 12 children ranged from 3 years 10 months to 14 years of age.

Capillary or venous blood was collected at 9.00 a.m., 4.00 p.m., 9.00 p.m., and the following day at 9.00 m. Serum as analyzed within one week of collection, and was stored frozen until that time. Acid-washed glassware was used for processing and storage. Serum iron deter minations are performed by modification of the micro method of Caraway (1) for Auto Analyzer (Technicon Chauncey New York) determination. The color was first developed by manual addition of reagents and the solution then introduced into the Auto Analyzer The re corded peak was compared to set of suitable standards All samples on each patient were analyzed simultaneously and in duplicate. The coefficient of variation of the deter minations was 5. %

The results (Table 1) are expressed both as micro-grams of serum iron per 100 ml and as the per cent relativ change from the 9.00 a.m sample to the later sample (difference in the re values divided by the average of the two values). The value of one standard deviation is given with each figure. In Table 2 the results are expressed as per cent decrease (difference between a.m. and p.m values divided by the m. value) in order to compare them with values available in the literature.

RESULTS

Analysis of the per cent relative change by the Student s t test reveals no significant difference from zero in the values of the group of younger children (Table 1). In the group of older children, there was a significant difference from zero of the relative change from 9.00 m to 9.00 p.m. (p 0.05). A significant d urnal pattern for the four samples was seen in 4 old.r children and

Table 1 *Serum iron levels during a 24 hour period*

Group	No.	9.00 m.	4.00 p.m.	9.00 p.m	9.00 m
Infants	12				
Serum iron, µg per 100 ml		114 50	113 ± 60	117 41	103 ± 35
Relative change		—	12 % ± 28	3 32	8 ± 24 %
Older children	12				
Serum iron, µg per 100 ml		93 ± 44	91 ± 25	71 ± 30	92 ± 41
Relative change		—	2 ± 31	−23 ± 39 %	4 % ± 18

Table 2. *Change of serum iron level from morning to evening*

Author	Age group	No. of patients	Decrease of iron level, %
Hamilton *et al.* (1950) (includes review of literature)	Adults	123	37.5
Laurell (1953)	Adults	10	31.5
Maurer (1952)	5½–13 years	19	26.6
Present report	2 weeks–20 months	12	None
	3½–14 years	12	25.6

one infant. A drop in serum iron level between the 9:00 a.m. and 9:00 p.m. samples was seen in 8 older and 4 younger children. The younger group had a higher mean level of serum iron and a wider range of values than did the older group reflecting the high values usually found in the early months of life. No child had levels indicating iron deficiency.

We were unable to find a correlation between physical activity or diet and the presence of diurnal variation of serum iron. The older children had longer periods of sustained nocturnal sleep. Many of them awakened briefly at night. The presence of brief waking periods did not appear to influence the serum iron values in the group of older children.

DISCUSSION

The diurnal variation of the mean level of serum iron in our two groups is compared with results from previous studies in Table 2. The findings in our older group are in agreement with those of Maurer (10). Normal adults have a greater and more consistent variation than do children. The absence of variation in the group of children up to 20 months of age is similar to the findings of Franks (3) in regard to plasma 17 hydroxycorticosteroids, where a significant diurnal pattern was first seen in the 3 to 17 year old age group. The similarity of patterns and age of onset of variation between serum iron and adrenocortical steroids does not indicate a control of serum iron variation by the adrenal or pituitary gland. Normal diurnal patterns of serum iron have been described in Addison's disease (4), Cushing's disease (8) and Simmond's disease (11).

The cause of the circadian rhythm of serum iron has not been clearly established, but may be related to the utilization of iron in red cell metabolism. Laurell (7) and Stengle (14) have noted a parallel between the levels of serum bilirubin and iron, indicating to them a variation in the rate of hemoglobin catabolism to account for the observed fluctuations. The diurnal variation is diminished in patients with erythropoietic disorders (12). Mauer (9) could not detect a diurnal proliferative activity pattern for erythroid bone marrow cells, although he was able to show decreased myeloid proliferation in the evening samples.

The periodicity of serum iron levels in adults is influenced by sleep patterns (6, 15). The lack of one sustained period of sleep in most infants probably contributes to their lack of daily variation in iron levels. In addition, the taking of naps by most young children may also influence the iron values. Alternating short periods of light and dark will abolish the periodicity of some physiologic functions in animals (5). Conditions in the hospital nursery and children's ward, where the normal variations of light and dark, noise and surrounding activity are not commonly found, may contribute further toward the damping of the variation of serum iron.

The age of onset of diurnal rhythms in children should be added to the many variables influencing serum iron levels. The lack of a diurnal variation of serum iron in infants less than 2 years old and the unpredictability of its occurrence in hospitalized children from 3 to 14 years of age must be considered when interpreting a value for diagnostic purposes.

SUMMARY

The diurnal variation of serum iron found in healthy adults was evaluated in two groups of children of different ages. No variation was found in 12 children aged 2 weeks to 20 months. In contrast, there was a significant variation of the per cent relative change of serum iron from a.m. to p.m. for a group of 12 children from 3 to 14 years of age. These results support the evidence for the late appearance of a biological "clock" in children.

ACKNOWLEDGEMENT

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CASE REPORT

IMMUNOGLOBULINS IN 13–15 TRISOMY SYNDROME DUE TO A TRANSLOCATION

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The 13–15 trisomy syndrome was first described by Patau *et al* in 1960 (13) Most children with this syndrome present a rather characteristic combination of malformations (16 17 18). The great majority of patients with this syndrome have 47 chromosomes with 13–15 trisomy Some of the affected cases, however have 46 chromosomes including a 13–15/13–15 translocation (3 5).

The present report concerns a male infant with the 13–15 trisomy syndrome due to a translocation. Special studies of serum immunoglobulins are reported.

CASE RECORD

Family history The mother born in 1939 and the father born in 1940, are both healthy There was no parental consanguinity The mother who has had three pregnancies, gave no history of abortions In 1963 she gave birth to girl, whose subsequent health and development have been normal. Her second pregnancy which resulted in the child described here, was uneventful Her third pregnancy in 1967 resulted in girl with small 6th digit on the ulnar side of the left hand. This infant is otherwise quite normal. No other instances of congenital malformations, mental retardation or other important defects or diseases have been recorded among the relatives.

Description of the patient. L. K., boy was born at term on October 12, 1965 The delivery was normal. His birth eight was 3270 g, height 48 cm and head circumference 33 cm. At birth and during the first 18 months of life the following abnormalities were noted.

Head. The skull was brachycephalic. In the parieto-occipital region the dura was exposed in 4 4 cm area through defect f the skin and bone (Fig. 1). This defect was repaired immediately after birth. Further abnormalities of the head included large nose with depressed nasal bridge and moderate micrognathia (Fig. 2).

Eyes. The eyes were set wide apart and the palpebral fissures were small (Fig 3). In both eyes, colobomata of the iris and choroid were seen. The lenses were small and that of the left eye was dislocated. When the boy was 2 months old dacryocystitis was noted on the right side and treated with probing and antibiotics The boy showed no conclusive signs of gaze fixation until 9 months of age. He could easily follow moving objects at 18 months of age. There was, however convergent strabismus.

Ears. The ears were low-set and malformed. The boy seemed to respond to sound from the ge of 7–8 months.

Heart In the neonatal period the heart was found to be displaced to the right. N murmurs were heard Electrocardiography revealed a delayed conduction corresponding to the left ventricle, and anti-clockwise rotation of the heart. No conclusive evidence of any other congenital heart defect was found during the period of observation.

Thymus. Repeated X-ray examinations of the chest showed normal outline of the thymus, corresponding to the age of the patient.

Abdomen and genitalia. Bilateral inguinal hernias were noted from birth and successfully repaired at the age of 6 weeks. A bean-sized supraumbilical hernia was also observed. The testes were undescended at birth but were brought dow into the scrotum at the hernia repair The penis was small and abnormally attached to the scrotum On X-ray of the abdomen, calcifications were found dorsally in the right upper quadrant. Intravenous pyelography gave normal results.

Extremities and skeleton. The boy had rudimentary 6th digits on both hands. The hands and fingers are kept in flexion, in way resembling the wrist-drop in

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Fig. 1. View of the back of the head at birth. Note the exposed dura in a 4×4 cm area in the parieto-occipital region.

radial nerve paresis. The plantar extension of the feet was limited. The heels were prominent. X-ray films of the skeleton revealed a flattened body of the tenth thoracic vertebra. The body of the fifth lumbar vertebra appeared to be split frontally.



Fig. 2. The patient a few hours after birth. Note the low-set malformed ear, the large nose and the rudimentary 6th digit on the right hand.



Fig. 3. Facial appearance of the patient at 3 months of age. Note the small palpebral fissures, the eyes set wide apart and the large nose with depressed bridge.

Skin. A persistent non-elevated capillary haemangioma was noted on the forehead, just above the bridge of the nose.

Development and neurology. At birth, a normal Moro response and normal sucking and grasp reflexes could be elicited. The boy was, however, hypotonic and also less active and alert than normal new-borns. During the following months his development was slow. At the age of 8 months he was unable to keep his head steady but tried to turn it around on sound stimulus. He could neither grasp intentionally nor turn around in the recumbent position. At 18 months of age he could roll from prone to supine and from supine to prone but could still not sit, crawl, walk or speak any single words. When held in a sitting position he could only hold his head up for a few seconds. No seizures are seen but he did have breath-holding spells. At echoencephalography a normal midline echo was found. The lateral ventricles were not enlarged. An electroencephalogram was considered to be normal for his age.

Chromosome studies. Karyotypes were obtained from cultured leucocytes from the patient and his parents. The technique employed was a modification of the method of Moorhead *et al.* (12). The results are summarized in Table 1. A total of 62 metaphase plates from two independent blood cultures of the patient showed 46 chromosomes. There were only five large acrocentric chromosomes and an extra large metacentric chromosome equivalent in size to a No. 3 chromosome (Fig. 4). All cells from the patient showed this anomaly. The relative length of the extra metacentric chromosome corresponded to twice the length of the long arm of a chromosome in group 13–15. Both parents had normal karyotypes.

Haematology. Routine blood tests gave normal results.

Table 1 *Summary of chromosome analyses of the patient and his parents*

	Chromosome counts			Karyotype interpretation
	45	46	47	
Patient				
Blood I	—	32	—	13–15/13–15 translocation
Blood II	—	30	—	13–15/13–15 translocation
Mother				
Blood	—	27	—	Normal karyotype
Father				
Blood	—	38	—	Normal karyotype

On starch gel electrophoresis of the haemoglobin, no fractions besides Hb A, F and A_2 were found. The Hb F concentration, determined according to Singer *et al.* (15) was 55%, 54%, 43% and 18% at the age of 8 weeks, 3 months, 5 months and 15 months, respectively. The Hb A_2 concentration was 0.5% and 1.1% at 8 weeks and 15 months, respectively. On the average, 33% of the polymorphonuclear leucocytes had two or more hook-like appendages of the type described by Powars *et al* (14).

Immunological studies. Quantitative determinations of the different immune globulins in serum were made by single radial diffusion in gel according to the method of Mancini *et al.* (11). Subnormal concentrations of IgG, IgA and IgM were found and IgD was never demonstrated (Fig. 5). One injection of polyvalent typhoid vaccine was given at the age of 4 months. This coincided with a rise in IgM level. However, no typhoid antibodies were detected in the serum. A booster dose of the typhoid vaccine was not given since the first injection caused a severe reaction. The patient was given 0.5 g of commercial gammaglobulin at the ages of 2, 3 and 6 months. This preparation contains mainly IgG and trace amounts of IgM. Blood smears were studied repeatedly and the lymphocytes were counted. At the ages of 1 week, 1 month and 3 months the lymphocyte counts were 6000, 10,000 and 11,000/mm³ respectively. A test of delayed cutaneous hypersensitivity was planned but it was not performed, since cutaneous hypersensitivity developed spontaneously to the plastic material of the splints used for correcting the hand and foot deformities. The skin healed after changing the material of the splints.

Infections. During the whole period of observation innumerable infections were noted, mostly upper respiratory infections and conjunctivitis. The boy also suffered from repeated diarrhoeas. This was thought to be due to bacterial and fungal infections, but stool cultures gave no confirmation. No sepsis, pneumonia, meningitis, otitis or pyelitis were ever diagnosed. Throat cultures yielded coli and staphylococci. Monilial Candida overgrowth was never noted and no evidence of Pneumocystis Carinii infection was found clinically or by chest X-ray.

Other investigations. The fasting blood glucose concentrations were normal. Two-dimensional paper chromatography of aminoacids in the urine gave a normal pattern.

DISCUSSION

The extra large metacentric chromosome in the patient, resembling a chromosome No. 3 has probably originated through a reciprocal translocation between two chromosomes of the 13–1

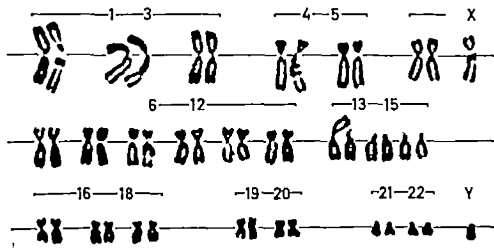


Fig. 4. Karyotype from the patient. There are 46 chromosomes, including 13–15/13–15 translocation chromosome (placed in the 13–15 group).

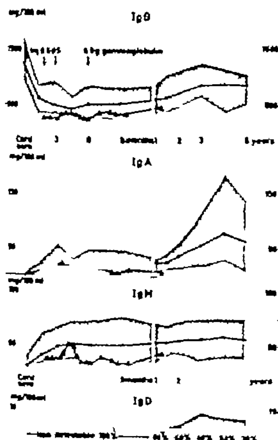


Fig. 5. The concentrations of the immunoglobulins G, A, M and D (mg/100 ml) Δ—Δ, compared with the results for healthy infants (shaded area).

group, with centric or near centric fusion. It is also possible that the extra chromosome is an iso-chromosome for the long arm of a 13–15 group chromosome. Thus extra genetic material equivalent to the long arm of a chromosome in group 13–15 was present. As the karyotypes of the parents were apparently normal the translocation (or isochromosome) found in the patient probably occurred during gametogenesis in one of the parents. Only two cases have been reported where a 13–15/13–15 translocation was transmitted to the patient by a parent (2, 6). In the other cases with this syndrome those parents who were investigated cytogenetically showed normal karyotypes (5).

From his clinical signs the patient under discussion should be classified as a case of 13–15 trisomy syndrome, although he lacked some of the features usually found in this syndrome such as apparent deafness, harelip, cleft palate and four finger lines. Our patient demonstrated the haemoglobin F elevation and the specific nuclear abnormalities of the neutrophils usually seen in patients with the 13–15 trisomy syndrome (7, 8, 10, 14, 19). Previously reported patients with this syndrome due to a translocation did not show any consistent clinical differences in comparison with the simple 13–15 trisomy cases with 47 chromosomes (1, 3, 19).

Our patient is still al[i]ve at 27 months of age which is long compared with most of the reported cases with the 13–15 trisomy syndrome. The majority of patients with this syndrome die in early infancy: the mean age at death being 10[?] days (17). Apnoeic spells, cardiac failure and bronchopneumonia are the most common causes of death (16).

The immunoglobulin concentrations found in our patient are low but not definitely abnormal when compared with the normal material of Johansson & Berg (9). Their normals, however, were selected among infants who had not shown any signs of infection during the immediate 2–3 weeks before the blood sample was obtained. Thus it seems justified to conclude that our patient was unable to respond to his repeated infections by increasing the IgG and IgM levels above minimum levels for healthy non-infected children of the same age. The IgA levels were normal during the first 6 months but subsequently diminished to abnormally low values. As far as could be determined, the patient had normal lymphocytes and an apparently normal-sized thymus. Cutaneous hypersensitivity, an unusual finding at this age, de[v]eloped rapidly.

These immunological observations are to our knowledge the first reported in a child with the 13–15 trisomy syndrome. The significance of our findings is difficult to evaluate until similar studies of other patients have been reported. The findings in our patient suggest, however, that the syndrome is associated with disturbed and, in some respects, delayed immunological development.

Many of the phenotypical deviations, such as mental retardation, retarded growth and dysplastic changes of the skeleton, found in patients with autosomal trisomy syndromes, have been ascribed to delayed embryonic development (4). The presence of embryonic haemoglobin (Gower 2) and increased amounts of haemoglobin Barts (γ_4) in

newborns with the 13–15 trisomy syndrome, as well as the delayed change-over from foetal to adult haemoglobin and the disturbed immunological development of our patient could also be consistent with the concept of a generally delayed maturation.

SUMMARY

A case of 13–15 trisomy syndrome, due to a translocation between two chromosomes of the 13–15 group, is described. The parents karyotypes were normal. The child showed typical features of the syndrome except for the absence of harelip cleft palate and four finger lines. Despite numerous infections the immunoglobulin concentrations were very low although not significantly lower than those found in normal, non-infected infants of the same age. At present the boy is 27 months old. Thus, he has survived longer than most other patients with this syndrome reported in the literature.

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CASE REPORT

TWO PATIENTS WITH A 46,XX,Er CHROMOSOME CONSTITUTION

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A ring chromosome represents a microscopically visible structural chromosome aberration, which is thought to arise through breakage in both arms of a chromosome followed by their end-to-end reunion. The occurrence of ring chromosomes, long been known in plant and animal material, has recently been observed in man in connection with various developmental abnormalities. Chromosomes of different groups have been affected, at least eight cases with rings in group E have been described (1 2, 3 5 6 7 9 10). In this communication two further patients with a ring chromosome in group E are presented. The cytological behavior of the ring chromosome of one of these patients has been reported earlier (4).

CASE REPORTS

The *younger patient* (T U) is an 11-year-old girl, born at term after normal pregnancy. Her birth weight was 2850 g. The mother was 22 years old at the time of delivery. She had one miscarriage previously. The patient has sister who suffers from congenital heart defect but is otherwise healthy.

The patient is small, being 110 cm tall and weighing 15.5 kg. Her skin shows patchy brownish discoloration with pigmented naevi on the trunk. The face is roundish with slight hypertelorism and the eyes have slight "antimongoloid" slant (Fig. 1). The mouth is wide and the teeth widely spaced. The skull is slightly brachycephalic with prominent forehead and small chin (Fig. 2). There are corneal leucomata in both eyes and the girl is also myopic. The external genitalia appear normal.

The psychomotor development of the patient has been slow from the beginning and she is now severely mentally retarded, her developmental age is about 8 months. The

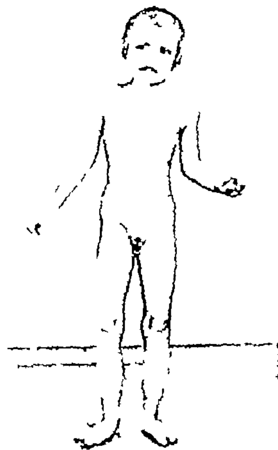


Fig. 1. The first patient, T. U.

A preliminary report of the patients has been given at the 18th Congress of Scandinavian Neurologists, Helsinki 1967 (11).



Fig Profile of the first patient.

movements of the patient are not well coordinated, the deep tendon reflexes are normal. The EEG shows diffuse abnormalities with absent alfa-activity and an abnormally slow background activity the girl has not had seizures. She behaves tranquilly cannot speak, cannot control her bladder or her bowels.

The urinary amino acids are normal. Intermittent ... of unknown origin has been noted. There have been episodes of laboured breathing for no known reason.

The second patient (P J) is 19-year-old girl. She was born at term after a normal pregnancy with birth eight of 3700 g. The mother was then 33 years old. T of her five children have died (one of malignancy and the other of peritonitis) while the others are healthy. One f the mother cousins is mentally retarded and has had seizures of unknown origin.

The patient is 138 cm tall and weighs 39 kg. She has somewhat masculine appearance with well developed muscles (Fig. 3). The skin is greyish-brown and there are numerous pigmented naevi (Fig. 4). The palate is high. Both retinae show numerous white spots (defined as retinitis punctata albescens), vision is normal. The fingers appear short. The external genitalia appear normal, the breasts are developed and the menstruation history is normal.

The patient learned to sit at the age of 7 months, to walk at the age of 14 months and to speak some words at the age of 4 months. She even ent to primary school, but had to leave because of poor success. Her Intelligence Quotient was later found to be 47. The patient is tranquil, cooperative, seeks contact with other persons and is able to perform simple practical tasks. She is toilet trained. From the age of 8 years she had seizures of grand mal type, which appeared about once in two months.

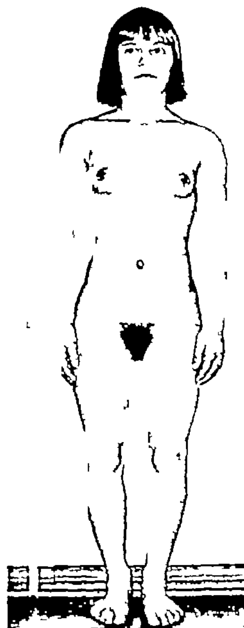


Fig 3 The second patient, P J

With medication she has been essentially free of these during the last two years. The EEG shows diffuse abnormalities with an abnormally slow background activity. In addition, slow 3–4 cps rhythm is noted occipitally and posttemporally predominantly on the right.

Dermatoglyphics

The dermatoglyphics of the hands are shown in Figs. 5 and 6. On soles of the feet the first patient



Fig. 4 Profile of the second patient.

has a distally opening loop in the first interdigital area on both sides and an additional loop at the base of the fourth toe on the left side. The second patient has a vestigial loop at the bases of the third toes, and in the hallucal area an additional fibular loop

Cytogenetic studies

Peripheral leukocytes were cultivated for 3–4 days according to a modified technique of Moorhead *et al.* (8). The methods differed slightly in the two cases, because the chromosome studies were made at different laboratories. For the autoradiography tritiated thymidine (The Radiochemical Centre, Amersham, England) was used.

Both patients were sex-chromatin positive and had a 46,XX,Er chromosome constitution. The ring chromosome replaced most probably one of the homologues of the pair 18 (Fig. 7). In the first patient this was further verified by autoradiography. The parents and sister of the first patient as well as the parents of the second patient had normal karyotypes.

In most cells the ring appeared monocentric and was quite uniform in size in both cases. It was missing in 13 % of the cells of the first patient and in 1% of the cells of the second patient (3 days culture). These cells thus had only 45 chromosomes. After four days culture the percentage rose to 10% in the second patient.

In both cases abnormal chromosomal material was seen in a few cells in which the ring was absent or defective. This granular material (Fig. 8) was interpreted as the rests of a disintegrating ring chromosome. In addition, micronuclei were seen in low frequency (4.0%) in the cells of the first patient (Fig. 9). These micronuclei probably originate from lagging ring chromosomes.

In addition to the monocentric ring, larger rings with two or more centromeres were observed in both cases in low frequency (Fig. 8). Cells with two or even three dicentrics were also seen, the chromosome numbers then being 47 and 48.

The variability in the appearance of the ring in the two patients is shown in Table 1.

DISCUSSION

Phenotypes associated with the Er chromosome have differed considerably from each other in the previously cases described (for review see Palmer *et al.* (2)), although in most patients the chromosome number 18 has been thought to be affected. Even the present two patients differ clinically in many respects, despite mental retardation, retarded growth, abnormalities in the EEG, eye defects and pigmentation defects, which seem to affect both of them. The second patient especially presents with only a few features suggesting a chromosomal etiology. As Lejeune *et al.* (6) has already noted, it scarcely seems justified to speak about a common "ring 18 syndrome" on the basis of the information available.

During the formation of a ring chromosome some of its genetic material is lost. A ring chromosome thus represents a deletion in the genetic sense. It is obvious that it is the quantity

Fig 5. The dermatoglyphic patterns of the first patient. Note the position of the axial triradii and the loop-patterns in the hypothenar areas. Three of the fingers have an arch, most of the others very shallow loops.

Fig 6. The dermatoglyphic patterns of the second patient. The axial triradii are somewhat distally displaced. I the interdigital spaces there are two distal loops on both hands.

PAT P.J. 46 XX Er

A 1–3 B 4–5

C = 6–12

D 13–15 E = 16–18

F 19–20 G 21–22 X X

Fig. 7 Karyotype of the second patient.

of this lost material and its site of origin in the chromosome that determines the genic constitution of the ring. It is no wonder then, that even apparently similar ring chromosomes may lead to different clinical syndromes, as is the case within the group of 18-ring patients presented. This mechanism has been considered especially by de Grouchy *et al* (2), who have studied the possible

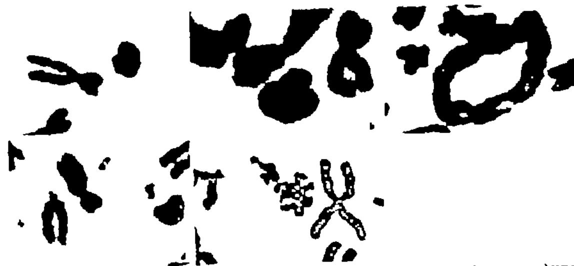


Fig. 8 Behavior of the ring chromosome. Second patient (*a*) Normal monocentric ring, (*b*) dicentric ring, (*c*) and- ucentric giant ring, (*d*) and ( ) disintegrating chromosomal material of ring

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CASE REPORT

YERSINIA ENTEROCOLITICA AS A CAUSE OF ACUTE MESENTERIC LYMPHADENITIS

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Hässig was the first to isolate, in 1949 *Yersinia enterocolitica* from liver abscesses established at the autopsy of two fatal cases of septicemia (7). It has been isolated in the 1960s from the alimentary canal of pig, chinchilla, dog and hare (1 3 4) In Sweden, Carlsson *et al* described in 1964 acute mesenteric lymphadenitis caused by *Y enterocolitica* in a young man (2) After this, two similar cases were reported by Winblad *et al* (12) who established in their extensive studies that *Y enterocolitica* is a fairly common cause of mesenteric lymphadenitis and terminal ileitis (13).

The first strains isolated were called *Pasteurella pseudotuberculosis* (synonyme of *Yersinia pseudotuberculosis*) (3 7) Daniels, in 1963 drew attention to the fact that they differed distinctly enough from the bacterium mentioned to constitute a species of their own, *Pasteurella X* now called *Y enterocolitica* (3 6) This as well as *Y pestis* and *Y pseudotuberculosis* are now suggested for inclusion in the family Enterobacteriaceae (6 11)

Two cases of acute mesenteric lymphadenitis in children caused by *Y enterocolitica* have been diagnosed at Aurora Hospital in Helsinki. Both patients were operated on for suspected appendicitis. As pediatricians and surgeons are sure to encounter cases of this kind we decided to publish a short review of these two cases.

CASE REPORT

Case 1

Hospital record 170154 The patient, girl aged 11 years, was brought to hospital on February 26, 1965 after developing fever and abdominal pain six days earlier She was better for couple of days, but on February 26 the abdominal pain began again. There was no vomiting On admission to hospital, marked tenderness on palpation was observed at McBurney's point. The axillary temperature was 36.8°C, the blood white cell count was 11100. Operation was decided on for the diagnosis acute appendicitis. The appendix was found to be very slightly reddish. In contrast, there was above the cecum thumb-tip sized, slightly yellowish lymph node which was excised and sent to the laboratory for bacterial culture. The girl recovered without complications and was discharged from hospital five days postoperatively

Bacterial culture of the excised lymph node grew in two days at room temperature and at +37°C grey-white, shiny smooth-edged nonhemolytic colonies, 0.5–1 mm in diameter Gram-negative rods were seen on Gram staining, short rods and coccoid forms were observed on staining with methylene blue. They stained bipolarly Short rods which moved slowly like tadpoles were seen in the bouillon culture at room temperature. The biochemical reactions were as follows: glucose + (acid), mannitol + lactose – saccharose + H_2S – urea + rhamnose – adonitol – indole – The strain agglutinated weakly on glass slide with Y enterocolitica rabbit immunserum kindly supplied by Prof S. Winblad. It proved to be guinea pig apathogenic when injected intramuscularly for two guinea pigs. Its antibiogram determined by Ericsson's method (5) was: penicillin 0, meticillin 0, ampicillin + sulphonamides + + streptomycin + + + tetracycline + + + chloramphenicol + + + erythromycin 0, lincomycin 0, gentamycin 0, cephaloporin 0, novobiocin 0, kanamycin + + + colimycin + + + polymyxin + bacitracin 0 neomycin +

The results of the antibody studies with the patient serum were as follows: on February 27 1965 agglutination titre with Winblad' *Y enterocolitica* strain (0-antigen) 20 and with *Y pseudotuberculosis* type I –

Case 2

Hospital record 241154. The patient was schoolboy aged 12 who had been operated on for adenoids five times and on December 2, 1966, his tonsils were removed. On December 28, 1966, abdominal pain and severe diarrhea

ed in. The diarrhea lasted for few days and was brought under control by fasting. The abdominal pain began again on January 1 1967 and gradually gained in severity. The patient's temperature rose to 38.2°C. A physician was summoned and he sent the patient to hospital with the diagnosis acute appendicitis. Mild tenderness was observed on palpation at McBurney's point. The axillary temperature was 37.4°C, rectal temperature 37.9°C, leucocytes 12700. On operation, fairly abundant clear fluid as seen in the peritoneal cavity. The appendix as innocent. Numerous small lymph nodes were established in the mesentery and one of them was removed for bacterial culture for Pasteurella. The operative diagnosis was mesenteric lymphadenitis. The patient made good recovery and as discharged from hospital five days postoperatively. In autumn 1967 his mother reported that he had had abdominal pain, fever and diarrhea again after the operation. The family had no domestic animals.

On bacterial culture, strain grew from the excised lymph node. Its properties proved to be identical with those of the strain isolated from case No. 1 and th the *Y. enterocolitica* of Winblad. Virus cultivation from the patient lymph node and stool sample as negative.

The results of the antibody study with the patient serum were as follows: on January 17 1967 agglutination titre with Winblad' *Y. enterocolitica* (0-antigen) 1280 and with *Y. pseudotuberculosis* type I –.

COMMENTS

These two cases were similar in their clinical features to the case of acute lymphadenitis in a boy aged three years who had been treated at Aurora Hospital (8). His lymphadenitis had been caused by *Y. pseudotuberculosis*. A difference from typical acute appendicitis was the longer duration of the disease, 5–6 days, and diphasic course. As such acute abdominal symptoms caused by Yersinia bacteria are obviously not rare more frequent etiologic studies should be performed. In addition to Winblad's results the commonness of the disease is indicated by the fact that three cases were diagnosed bacteriologically in the course f two years at Aurora Hospital although the number of specimens examined per annum was only some 20. There is no information in the literature concerning the duration of immunity after the infection.

It may be mentioned that the Yersinia strain which was isolated in 1965 was at first regarded as a variant of *Y. pseudotuberculosis* until W blad's strain of *Y. enterocolitica* was received. The serological antibody studies on the patient concerned support the view that there are different serotypes of *Y. enterocolitica*. Frederiksen demonstrated three different 0 antigens, most strains belonged to one serotype only (6). U o the present, eight partial 0-antigens ha been demonstrated by Winblad (14). In h n study at least one 0 antigen was found c mmon to all 28 *Y. enterocolitica* strains (10). *Y. enterocolitica* can be distinguished umo from *Y. pseudotuberculosis* on the basis of the following characteristics: saccharose + (*Y. pseudotuberculosis* –), rhamnose and adonitol – (*Y. ps.* +) apathogenic to guinea pig (*Y. ps.* pathogenic to guinea pig) (9).

SUMMARY

Two cases of acute mesenteric lymphadenitis caused by Yersinia enterocolitica in children aged 11 and 12 years are described. At appendectomy the appendix was found innocent, but in the ileocecal region and in the mesentery there were lymph nodes from which bacterial culture grew *Y. enterocolitica*. In one patient, agglutinins against *Y. enterocolitica* were established in the titre 1:80 and in the other in the titre 70.

The importance of etiologic studies in cases of acute mesenteric lymphadenitis is emphasized.

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CASE REPORT

MENINGITIS CAUSED BY *NEISSERIA CATARRHALIS*

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Nonpathogenic *Neisseria* are encountered quite frequently in the nasopharinx and in the upper respiratory tract. In man reports on a well established pattern of pathogenicity are rare. A survey of the literature on meningitis reveals a very small number of cases.

The following report describes a case in which a clinical picture of meningitis developed and only *Neisseria catarrhalis* was cultured from spinal fluid and throat specimens.

CASE REPORT

Stefania C., 14-month-old female infant, was brought to the hospital June 18, 1967 24 hours after she developed symptoms of vomiting and fever (39°C) and 6 hours after petechiae were noticed on her trunk. The patient history was negative.

Physical examination revealed the presence of numerous petechiae spread on the trunk and arms, and redness of pharynx. She had temperature of 39°C and pulse-rate 130. Disturbances of sensorium and prostration were present.

Neurologic examination revealed symptomatology of meningeal irritation: the neck was stiff Kernig and Brudzinski signs were present.

At the time of admission the patient weighed 12 kg The white blood cell count was 9600/mm³ with 70% neutrophils, 26% lymphocytes, 2% monocytes and % eosinophils Hb 84%. Other blood examinations showed normal values of serum electrolyte balance, glycemia and azotemia. The electro and immunoelectrophoresis of plasma proteins revealed nothing abnormal C-Reactive protein test + X-rays of the chest showed clear lung fields without radiographic evidence of pneumonitis

The cerebrospinal fluid examination revealed purulent fluid, 3+ Pandy reaction, 70 mg/100 ml protein, 30 mg/100 ml sugar and 1150 cells/mm³ of which 75% were polymorphonuclear leukocytes and 15% lymphocytes. A gram-stain of the spinal fluid smear showed moderate number of intra and extracellular gram-negative diplococci.

Bacteriologic study

Spinal fluid cultures were incubated at 37°C on blood-agar Growth occurred after 24 hours. The colonies were of about 1 mm in diameter convex, bluish-grey with glistening surface. Gram-stain of the colonies revealed pure culture of gram-negative diplococci, occasionally occurring in tetrads (Fig. 1), which produced positive oxidase and catalase reactions. The results of the first isolation were confirmed by inoculating the organism on nutritive agar and heated blood-agar at room temperature. On subcultures in Loeffler blood serum slant at 37° and 22°C the colonies failed to produce pigment after 4 days of incubation. Anaerobic cultures were made and yielded no growth after ten days. Phenol red rabbit serum broth (15) adjusted to the pH 7.2 was used as the base medium for the carbohydrate fermentations. Glucose, lactose, maltose and sucrose were not fermented. These characteristics correspond to those of *Neisseria catarrhalis* (3). In *in vitro* disk-diffusion tests the microorganism was found sensitive to erythromycin, chloramphenicol, tetracyclines, novobiocin, penicillin G and methicillin. The same organism grew from throat culture

Progress and treatment

The infant was treated with daily dosage of 4 million units of penicillin G, erythromycin and chloramphenicol 25 mg kg of body weight each, 25 mg of hydrocortisone succinate given intravenously: methicillin 1 g and adrenal extract 1 ml given intramuscularly and phenobarbital sodium 30 mg given orally The patient improved and became afebrile after 3 days of treatment, the signs of meningeal irritation completely disappeared Treatment was continued until the sixth day when the intravenous therapy was discontinued The intramuscular treatment was maintained and 1.5 mg of dexamethasone was added. A control spinal fluid examination carried out at the eighth day showed normal values. On the 22th day the patient was discharged as recovered. Three months later she was reported as being in good health.



Fig 1 Blood-agar culture of *Neisseria catarrhalis* isolated from the spinal fluid.

COMMENT

The genus *Neisseria* can be divided into pathogenic and nonpathogenic groups. The pathogenic *N gonorrhoeae* and *N meningitidis* grow on blood-agar at 35–37 C, nonpathogenic species grow at 22 C on ordinary media. According to *Bergey's Manual* (3) nonpathogenic *Neisseria* are divided into two major groups, the chromogenic and the nonchromogenic species which include *N catarrhalis*. This aerobic nonchromogenic diplococcus was first isolated and described by Ghon & Pfeiffer (7).

Table 1 *Meningitis due to Neisseria catarrhalis*

Age	Sex	Outcome	Case reported	Authors
3 months	—	Fatal	1	Wilson (1908)
31 days	F	Fatal	1	White (1917)
6 months	—	Recovery	1	Mayerhofer (1918)
5 yrs	F	Fatal	1	Garland (1923)
31 yrs	F	F tal	1	Moersch (1928)
30 yrs	F	Fatal	2	Moersch (1928)
35 yrs	M	Fatal	1	Gaup (1933)
—	—	Recovery	1	Zoeller (1933)
4 / yrs	M	F tal	2	Zanke (1936)
1 / yrs	F	Fatal		Zanke (1936)
5 months	F	Recovery	1	Bergquist (1940)
45 yrs	F	Fatal	1	Newing (1947)
4 yrs	F	Recovery	2	Sevini (1957)
5 yrs	F	Recovery		Sevini (1957)
13 yrs	M	Recovery	1	Bentegeat (1960)
2 / yrs	M	Recovery	1	Pfister (1965)
14 months	F	Recovery	1	Cocchi (1967)

The case here presented may be considered a meningitis due to *N catarrhalis* according to the bacteriologic examination of the cerebrospinal fluid and the clinical picture. The criteria for diagnosis of species depended on the cultural characteristics and biochemical activities of the microorganism. In our patient, *N catarrhalis* was also cultured from the throat, suggesting that the organisms invaded the bloodstream and then the cerebro-spinal fluid.

A survey of the literature revealed only 17 cases of purulent meningitis caused by *N catarrhalis* (1 2, 6 8–14 16–19). Table 1 summarizes these cases. Meningitis caused by this ordinarily saprophytic organism is very rare, but very serious infection. The clinical picture resembles meningitis due to the closely related *N meningitidis*. The petechiae which accompany meningitis are the same as those occurring in meningococcal septicemia.

N catarrhalis meningitis is especially prevalent in children. Female predominated in a 10 to 4 ratio for cases in which the sex was given. Eight patients recovered and ten died. The previously reported cases indicate that the effect of antibiotic therapy varied from case to case. Bentegeat *et al* (1) report that the organism revealed it to be *in vitro* sensitive to tetracycline erythromycin, novobiocin and polyxin. Improvement, however was reached only when polymyxin was given intrathecally. In the case reported by Pfister *et al* (13),

excellent clinical response was obtained with chloramphenicol, penicillin G and sulfisoxazole. In our case the patient improved very quickly after penicillin, erythromycin and chloramphenicol therapy This was in accordance with the *in vitro* sensitivity tests.

Meningitis due to ordinarily saprophytic microorganisms is probably more common than the small number of cases reported in the literature suggests (4 5 13). In many cases of meningitis attributed to genus *Neisseria* there may have been a failure to identify the organism with absolute certainty The importance of exact identification is not only a scientific duty but a therapeutic need dependent on the varying sensitivity to antibiotics of *Neisseria*.

SUMMARY

A case is reported of meningitis due to *Neisseria catarrhalis* in a 14-month-old infant. The organism was cultured from the spinal fluid and throat. The patient recovered after penicillins, erythromycin and chloramphenicol therapy

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CASE REPORT

A SWEDISH FOETAL HAEMOGLOBIN VARIANT

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Beckman *et al* (1) in 1966 described a new haemoglobin variant, which they provisionally called Hb Uppsala. The variant was found in six apparently healthy members of a Swedish family. In two of these persons Hb Uppsala could be quantified and in both of them the relative concentration was 29%. Hybridization experiments indicated that the abnormality of Hb Uppsala was located in the α-chain. We have studied an infant boy, son of the propositus, from birth up to the age of six months.

METHODS

Stroma-free haemolysates were prepared by ultracentrifugation and subjected to starch-gel-electrophoresis using a Tris-EDTA-borate buffer pH 8.6 (4). Inclusion bodies in red cells were searched for after 30 minutes incubation of the cells with 1% brilliant cresyl blue in 0.9% saline. Red cell counts were performed electronically by "Celloscope". Otherwise standard methods were used.

CASE HISTORY

R. S. was the first son of a 23-year-old apparently healthy woman who was known to have the abnormal haemoglobin. Her pregnancy was uneventful and the delivery occurred at term. On physical examination the boy appeared normal. He weighed 4040 g. The haematocrit of the cord blood was 55%. The boy was examined repeatedly during his first week of life. Nothing abnormal was found and there was no jaundice. At four days of age, the plasma bilirubin concentration was 1.8 mg/100 ml. Haematological studies are reported in Table 1 together with some data concerning his mother.

During the following six months, the boy's development was normal. At four and a half months, microcytosis was noted (Table 1). The iron content of his food at this age was estimated as 10 mg daily. Twenty-five mg of ferrous iron was given each day in divided doses for a period of six weeks. No change of the mean cell volume was noted, however.

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Table 1 *Haematological data of mother and child*

Iron (25 mg Fe) was given daily between days 132 and 173

	Mother at delivery	Child (age, days) 1	4	41	90	132	173
Haemoglobin, g/100 ml	12.6	17.1	17.9	15..	12.2	11.8	11.9
MCV μ^3	87	122	—	91	87	76	73
MCH, g 10^{-12}	28	38	—	33	27	26	25
MCHC, %	32	31	36	36	31	35	34
Reticulocytes, %	1.8	4.8	7.4	0.6	3.0	2.2	1.0
Inclusion bodies	0	0	0	0	0	0	0
Platelets	180,000		230,000		420,000		
Leucocytes	7,900		6,700		12,200		

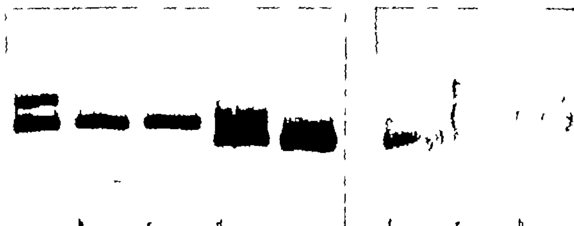


Fig. 1. Starch gel electrophoresis of red cell haemolysates. From left to right: (*a*) The mother (Hb A_2 + Hb A + Hb Uppsala), (*b*) and (*c*) normal adults (Hb A_2 and Hb A), (*d*) the patient, cord blood (Hb F + foetal Hb Uppsala), (*e*) cord blood from normal newborn (Hb F + Hb A), (*f*) cord blood from normal newborn (Hb F + Hb A), (*g*) the patient aged 4 months (Hb A_2 + a little Hb F, Hb A + Hb Uppsala), (*h*) normal adult (Hb A_2 + Hb A). (*a*) to ( ) refer to one electrophoretic run, (*f*) to (*h*) to another run.

HAEMOGLOBIN STUDIES

In cord blood, an unknown fast component was found together with a slower component which migrated as fast as normal Hb F. The fast component migrated faster than Hb A but slower than Hb Uppsala of the mother. Small amounts of Hb A and a small fraction migrating like Hb Uppsala were also seen but no Hb A_2 (Fig. 1). The two main fractions—that migrating as Hb F and the unknown fraction—appeared to have approximately the same concentration. At *one week of age* the same haemoglobin fractions were seen. *At six weeks of age* the Hb A and Hb Uppsala fractions appeared to have increased in concentration. The unknown fast component was still seen.

At *three months of age* the unknown fast component had disappeared. Four fractions were seen, one migrating slowly like Hb A_2, two moving like Hb F and Hb A and one fast, moving like Hb Uppsala. *At four and a half months of age* the same four fractions were seen although the Hb F was much less prominent. The changes in the haemoglobin electropherogram are shown schematically in Fig. 2.

DISCUSSION

The unknown fraction now found migrated faster than Hb A but slower than Hb Uppsala. It was present in large amounts in the cord blood and in the blood drawn when the boy was one week old. After that time, the relative concentration of the fraction decreased. In the blood sample obtained at three months, it had disappeared. The ratio between the relative migration velocities of Hb F and the unknown fraction was similar to that between the velocities of Hb A and Hb Uppsala. The findings indicate that the fraction is a foetal type of the Uppsala haemoglobin.

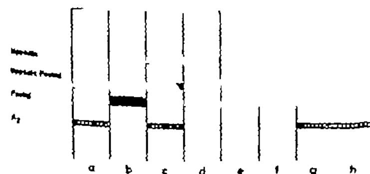


Fig. 2. Schematic description of the electrophoreses. (*a*) = Normal adult, (*b*) = cord blood from normal infant, (*c*) = the mother, (*d*) to (*h*) blood from the patient, (*d*) = cord blood, (*e*) = age 1 week, (*f*) = age 6 weeks, (*g*) = age 3 months, (*h*) = age 4 ½ months.

The hybridization experiments performed by Beckman *et al* (1) showed that the abnormality of Hb Uppsala was located in the α-chain. The haemoglobin could then be designated $\alpha_2^{Uppsala}\beta_2^A$. An additional abnormal fraction found in the haemolysates of these individuals was thought to be the Uppsala variant of Hb A_2, i.e. $\alpha_2^{Uppsala}\delta_2^A$. It seems logical that the unknown fraction now found should have the composition of $\alpha_2^{Uppsala}\gamma_2$.

With the α-chain abnormality in adult individuals there are no signs of disturbed erythropoiesis (1). The present study indicates that the synthesis of the foetal type of Hb Uppsala, also, does not interfere with red cell production and does not provoke hyperhaemolysis during the neonatal period. Hb F and the foetal type of Hb Uppsala were found in approximately equal amounts in cord blood. The findings indicate that the foetal type of Hb Uppsala is not synthesized after birth since it was not visible at the 3 months-examination. In contrast Hb F synthesis must have continued after birth since it was still evident at the $4\,^1/_2$-months-investigation. This is in accordance with studies of normal infants (2). A pronounced inhibition of the synthesis of normal α-chains might conceivably be connected with the appearance of Hb H and Hb Bart's. Neither of them was seen at electrophoresis, however, and no H inclusion bodies were found.

At $4\,^1/_2$ months of age, the boy had small erythrocytes (MCV 76 μ^3) and six weeks later the cells had the same volume (73 μ^3). The haemoglobin concentration was normal, however (MCHC 35 and 34 %). Although the normal variation of the cell volume in infancy is not well known, it may be noted that Moe (3) found only four 3 month old infants out of 171 studied who had cells smaller than 80 μ. These infants, as well as the present one, were given an adequate amount of iron in their food. The microcytosis of the present case is remarkable and may be a special feature of infants with the Uppsala haemoglobin variant.

SUMMARY

The infant boy of a Swedish woman with the haemoglobin variant Hb Uppsala, was studied during his first six months of life. In cord blood, two major haemoglobin fractions were seen at electrophoresis. One behaved like normal foetal haemoglobin and the other migrated faster than Hb A but slower than Hb Uppsala. When the boy was three months old, this unknown fraction had disappeared and Hb Uppsala was found. Since the abnormality of Hb Uppsala is known to be located in the α-chain, the unknown fraction was considered to be the foetal type of Hb Uppsala.

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PROCEEDINGS OF PEDIATRIC SOCIETY

DANISH PEDIATRIC SOCIETY

Meeting Oct. 11 1967

A. Ersler *Erythropoiesis after splenectomy*

Erythropoiesis is regulated by a hormone, erythropoietin, by means of a feed-back system. Decrease in erythrocytes and the resulting decrease in haemoglobin level cause decreased supply of oxygen to the tissues which results in increased production of erythropoietin in the kidneys which stimulates the bone-marrow to increased production of erythrocytes.

Following splenectomy in spherocytosis a decrease in reticulocytes occurs and thus cannot be explained as a result of previous increase i erythrocytes on account of pre-operative transfusions and emptying of the spleen immediately prior to ligation of the vessels as this decrease in reticulocytes also occurs when these procedures are omitted. A pronounced decrease in serum iron is, however observed as the spleen contains great quantities of iron and the resulting iron-deficiency is considered to explain the decrease in reticulocytes.

Discussion

S. Killmann. What happens if iron is administered after splenectomy?

A Ersler The condition is comparable to iron-deficiency in chronic disease despite administration of iron as the iron is bound in the reticulo-endothelial system.

S. Killmann. The transferrin level is low in chronic disease but it is normal here.

A. Ersler The decrease in the transferrin level occurs gradually

P H Brestrup How long does the low serum iron persist?

A Ersler For up to 3–4 weeks.

C G Bergstrand. Has an increase in the thrombocyte count been observed?

A Ersler No.

B Zachau-Christiansen. What does staining of the bone-marrow for iron reveal?

A Ersler Quantities of haemosiderin.

S Killmann. Was the bone-marrow followed as regards erythropoiesis?

A Ersler Before operation the nucleated red blood cells comprised 50–60% of the cells and after operation 15–20%

Henning Andersen Testosterone is known to be of significance in erythropoiesis. Was any difference observed between children/adults and males/females in this material?

A Ersler Not among these patients.

B Friis-Hansen. Why was the erythropoietin titre so low before splenectomy and why was no increase observed?

A Ersler It is difficult to answer It is known, however that the erythropoietin titres remain unchanged when the hæmatocrit values are over 20 and only increase when hæmatocrit values are lower

M Yssing, S. Jarnum & H. Jensen. *Protein metabolism in seven children with the nephrotic syndrome investigated with ¹ I-albumin ¹ I-IgG and ⁵⁹Fe-imferon*

In order to elucidate the question of the quantitative role of gastro-intestinal protein loss in the protein metabolism in nephrotic children, studies of the albumin and IgG metabolism with parallel investigations for the gastro-intestinal protein loss were undertaken in seven children aged 2–11 years with untreated nephrotic syndrome.

The protein metabolism was investigated by means of a double tracer technique employing ^{131}I-albumin and ^{125}I IgG. As a measure of the gastro-intestinal protein loss, symbolised by faecal excretion of macromolecules, the ^{59}Fe-imferon test was employed.

Expressed in relative values, increased metabolism of albumin and IgG in the organism were demonstrated in all of the children. This can only partially be accounted for by the renal protein loss. Four patients exhibited abnormal gastro-intestinal protein loss expressed by increased ^{59}Fe imferon faecal excretion. In three patients, the results of the ^{59}Fe-imferon test were within normal limits.

By comparison with corresponding investigations undertaken in patient groups with known protein-losing gastro-enteropathies it appears probable that the gastro-intestinal protein loss in all seven children with the nephrotic syndrome investigated did not play any quantitative part in the total protein metabolism of these patients and thus was not an etiological factor in the increased endogenic protein hypercatabolism demonstrated in these patients.

Discussion

P A Krasilnikoff: In Glostrup Hospital we have investigated albumin metabolism employing ^{131}I albumin in a girl, aged 3 ³/₄ years suffering from the nephrotic syndrome. The method of investigation was identical with that described by Yssing *et al.*

The investigation was commenced after the patient had received prednisone for six days but before the effect of this treatment had commenced. Th proteinuria and oedema were thus unchanged and the weight was constant. On the eighth day of treatment the "fractional disappearance rate (FDR) was calculated by means of a ratio between the plasma-activity and the excreted activity in the urine. The FDR rate was found to be 111 % of the intravascular albumin mass. The fractional catabolic rate (FCR) = the endogenic breakdown and fractional proteinuric rate (FPR) comprised 70 and 41 % respectively of the intravascular albumin mass as FDR = FCR + FPR.

From the 9th–12th days of treatment, the proteinuria disappeared gradually the oedema diminished and the weight returned to normal. Simultaneously the patient s intravascular albumin mass increased from 4.5 g to 10 g. By means of mathematical analysis of plasma and whole body graphs, FCR was now calculated to be 7 4 % of the intravascular albumin mass. As the endogenic breakdown thus decreased by more than the intravascular albumin mass increased, this must imply that, simultaneously with the cessation of symptoms, the factor responsible for the increased endogenic breakdown under the nephrotic condition was removed.

M. Osler & B. Zachau-Christiansen. *Fractionated serum protein determinations in associated maternal and cord blood*

It is known that the total serum protein concentration in pregnancy decreases on account of decrease in albumin concentration even although the alpha-globulin fractions increase slightly and the beta-globulin concentrations increase considerably The concentration of gamma-globulin only alters slightly The total protein concentration in the foetus increases and appears to attain the same level as that in the mother in the mature infant. It has been demonstrated that the serum albumin concentration in the mature child is higher than that in the mother

By means of analysis of blood from 35 women in labour and of cord blood from their 37 infants (two sets of twins) all with birth weights of ≤2500 g (average 1810 g) the following concentrations (g/100 ml) of total serum protein, albumin, alpha-1 alpha 2 beta- and gamma globulin were found 6.5 3 6, 0.49 0.75 0.95 0.80 and 5 1 3 4 0.23 0.36, 0.44 0.65 The two mothers of twins had values which deviated even

more from the norm. 6.., 2.4 0.65 0.85 1.20 1.05

In nine infants who died during the first day of life or suffered from the respiratory distress syndrome, the serum protein concentration was 4.0 g/ml and the albumin concentration 2.8 g/ml. The remaining infants had values of 5.4 and 3.6 g/ml. The average birth weights for the groups were 1680 and 1880 g. Apart from the three infants with most pronounced jaundice, icteric infants showed lower values than non-icteric infants: 4.9–3.2 g/100 ml compared with 5.3–3.6 g/ml, birth weights of 1780 and 1820 g. The three most jaundiced infants in this limited material were not sensitized but had, nevertheless, an average birth weight of 2230 g and the total serum protein concentration was 5.8 g/100 ml and albumin concentration 4.0.

The 19 infants who had higher albumin concentrations than their mothers had an average birth weight of 1830 but, in this group, there was a preponderance of infants with birth weights which were lower than corresponded to the durations of pregnancy

The following regression equations between the infants protein fractions (y g/100 ml) and those in the mothers (x) were calculat   0.80 x – 0.02 (‡), $y = 0.62\ x + 1.30$ (‡)   – 0.16, $y = 0.20 + 0.24$ $y = 0.45\ x + 0.03$ 18 x + 0.50

The infants protein fraction   essed by the birth weight (x kg). $y = 0.9$ – 0.4 x + 2.58 (‡) $y = 0.004$ x – 0   – 0.18 (‡) $y = 0.1$ x + 0.2.., $y = 0.2$

In the equations marked with (‡)   rela tions are significant. The order in the   total serum protein, albumin, alpha 1   alp beta- and gamma globulin.

Discussion

J Melchior drew attention to previous total serum protein investigations in newly born infants   view of the value of administration of protein

C G Bergstrand In previous investigations, employing paper electrophoresis we found a protein fraction between albumin and alpha-globulin in foetuses but not in newly born infants. Was a similar protein observed in the smallest infants here?

B Zachau-Christiansen No

Meeting Nov 22, 1967

Leo Stern (The Montreal Children's Hospital, Canada): *Temperature regulation in the newborn.*

Meeting Dec 12, 1967

Christmas meeting with ladies.

E. Soerrason. *Bringing up children à la Emile*

Meeting Jan. 10 1968

N J Brandt. *Follow-up control of patients with hereditary galactosaemia and in particular measurement of galactose 1-phosphat*

Hereditary galactosaemia is caused by deficiency of the enzyme, galactose-1-phosphate-uridyl-transferase. When the diet contains galactose galactose 1-phosphate accumulates. This i toxic and results in the well-known clinical and secondary biochemical symptoms.

Treatment is simple and consists of a galactose free diet which can save the patient lif and f it is instit ted sufficiently early nd is carried out effectively can protect him from the late sequelae mental retardation, cataract and cirrhosis of the liver

It is important to submit the children t regular control examination in view of their general well being, mental development, liver function and

changes in the lenses. The most important biochemical control of correct diet is measurement of galactose 1-phosphate in the erythrocytes.

Galactose 1-phosphate can be measured by Guthrie's method but the technique does not appear to be sufficiently sensitive. The enzymatic technique (with galactose 1-phosphate-uridyl-transferase from normal individuals as the measuring enzyme) is relatively difficult and not suitable for routine use. A chromatographic technique has been evolved in which galactose-1-phosphate is isolated from the haemolysate on ion exchangers and after liberation is hydrolysed to galactose which is then determined semi-quantitatively by means of thin layer chromatography on Kieselgel G. The technique is sufficiently sensitive to determine whether the patients have over or under an arbitrarily chosen permissible value for galactose 1-phosphate viz., 2 mg per 10 ml erythrocytes. If the values exceed this level, the diet must be revised.

Discussion

N. Hobolth. How much blood is required?

N. J. Brandt: Approximately 2 ml heparinized blood.

J. Mølsted Pedersen: *Glucose tolerance in newly born children of diabetic mothers*

Fifty newborn infants of diabetic mothers and 60 of non-diabetic mothers were investigated by intravenous glucose tolerance tests 1–6 hours after birth.

After injection of glucose via the umbilical vein, blood samples were drawn at 10-minute intervals during the next hour and, as an expression of the glucose tolerance, the k-value for each infant was calculated. The infants in the diabetes group comprise 16 infants of non-insulin-treated mothers and 34 of insulin-treated mothers.

The k value increased with the birth weight not only in infants of diabetic mothers but also in infants of non-diabetic mothers. In each weight group the k-value was lowest in infants of non-diabetics mothers, highest in the insulin-treated group and in between in the infants of non-insulin-treated mothers.

By means of regression calculation between the birth weight and the k-value a strongly positive linear correlation was found between these values in the infants of non-diabetic mothers. The same holds also true for infants of non-insulin-treated diabetic mothers. Among infants of insulin-treated diabetic mothers, the relation between the k-value and the birth weight was less pronounced and not linear and there was great scatter of the k values in all of the weight groups. The average k value was, however, still significantly greater for infants with birth weights of over 3500 g than for the weight group under 3500 g.

Comparison of the fasting plasma glucose taken simultaneously from the umbilical vein, umbilical artery and heel from 28 infants of diabetic mothers, a statistically significant difference was found as the plasma glucose was, on an average, 9 mg/100 ml lower in the heel-blood not only in infants of diabetic mothers but also in control infants. No significant differences in the glucose values were encountered between the umbilical vein blood and the umbilical artery blood.

Twenty infants in the diabetic group and 23 from the control group were investigated further by intravenous glucose tolerance test, both immediately after birth and on the fifth day of life. The result of this investigation shows that on the fifth day the differences in the glucose tolerance both those attributable to birth weight and those attributable to diabetes were evened out.

The relationship found between the k values and the birth weight in newly born infants probably expresses the effect of a generalized growth factor which acts via the glucose-insulin system.

Discussion

P. Plum drew attention to the fact that there must be growth factors other than the hyperglycaemia.

V. Esmann. The increased glucose tolerance in infants of diabetic mothers may be expression of an increased deposition of glucose in the liver. In this connection, it may be of significance that glycogen synthesis and glucokinase are developed before birth while glucose expenditure from the liver via glucose-6-phosphatase, does not commence until in immediate association with birth and the subsequent hours (or days) thereafter. A modification of this regulation mechanism caused by the hyperinsulinism demonstrated deserves more detailed consideration.

E. Thamdrup: How early was feeding commenced?

L. Hobøel Pedersen. No nourishment was given during the first 4 hours. The blood sugar was frequently 0 within the first six hours.

I. Jørgensen: *Hepatomegalic glycogenosis*

Within the past 18 months we have seen two cases of hepatomegalic glycogenosis in Kolding: type VI in which the enzymatic defect is localised to the liver phosphorylase and type III in which the defect is localised to amylo-1 6-glucosidase, respectively.

The patient with type VI was a boy aged 4 1/2 years with considerable hepatomegaly increased abdominal venous markings, reduced growth in length, intermittent low fasting blood sugar values, metabolic acidosis and ketonuria. The serum GO-transaminases were moderately raised. The galactose and glucagon tests showed increase in blood glucose. The glycogen content of the liver was found to be 5.5% w/v. The diagnosis was confirmed by enzyme investigation on liver tissue and leucocytes. The patient was treated with supplementary protein for nine months and, during this period, the transaminase values became normal the growth in length was accelerated and the hepatomegaly disappeared. Investigation of leucocyte phosphorylase activity in the clinically healthy members of the family (V. Esmann, Arhus) revealed that the mode of inheritance was sex linked recessive. The disease has, however been described in patients of both sexes. It is possible that type VI can be subdivided into several groups according to the different defects in the phosphorylase activating system according to which each defect can be conceived to be controlled by an independent gene with its own particular mode of inheritance.

The patient with type III was aged 2 1/4 years. He had also hepatomega height was normal. Considerable b was found. The glucose tolerance abetic result. The intravenous gala ed a normal increase in blood glu on test in the fasting state did n in crease in blood glucose and a te increase was noted when the te e four hours after a meal. The diag firmed by enzyme nvestigation on and leucocytes. The activity of am cosidase in leucocytes from the pa paternal grandmother and maternal gr was investigated. The acti ty in the r found to be lowered but was normal other relatives examined. It is d fficult to an opinion about the mode of inh tan basis of this investigation. Both sex-l nked re heredity and dominant hered ty th poor pene a tion are possible. This pa ent wa also eat with supplementary protein to counteract the tend ency to hypoglycaemia. No defin te h g the clinical condition could be bserved t al

Discussion

V. Esmann described the principles f the analysis.

Vagn Andersen, Christian Koch, Rene Vej aar & Knud Wilken-Jensen. *Fatal e anulo disease. A d fect in neutrophile leucoc es* (Published in *Acta Paediat Scand* 5 110 1966)

Discussion

E. Thamdrup. Is allergy to foodstuff typical

C. Koch. No.

J. Krogelbach emphasized the similarity to Wiscott-Aldrich syndrome.

A. Schondel. Were there healthy siblings

C. Koch. Yes, but they wer not investigated

P. Petterson d

NEW BOOKS RECEIVED

G. Wolstenholme & M. O'Connor (eds.): *Health of Mankind* (Ciba Foundation 100th Symposium), 297 pp. J & A. Churchill Ltd., London 1967 60s

S. G. Clayton (ed.): *Obstetrics: Some Current Problems.* British Medical Bulletin, vol. 24 London 1968. 40s.

J Gerbeaux. *Tuberculose primaire de l'enfant* 284 pp. (with 51 figs.) Éditions Médicales Flammarion, Paris 1967 70 Fr

Y Brackbill (ed.): *Infancy and Early Childhood. A Hand-Book and Guide to Human Development* 523 pp. Collier-Macmillan Ltd., London 1967 84s.

ANNOUNCEMENT

II INTERNATIONAL SYMPOSIUM ON PEDIATRIC ALLERGY

This meeting will be one of the preliminary scientific -nts to the XII International Congress on Pediatrics. will be held at the Unidad de Congresos del Centro Médico Nacional del Instituto Mexicano del Seguro Social" on November 30 and December 1 1968 Further information by Dr. Luis Gómez-Orozco, Departemento de Alergia del Hospital Infantil de México, Calle Dr. Márquez No. 162, México 7 Distrito Federal, Mexico.

Acta Pædiat Scand 57 463–467 1968

ACTINOMYCIN D AND TRANSVERSE LINES OF GROWING BO

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Transverse lines of increased density are often found in radiographs from the terminal segments of growing bones of infants and children. It has been generally assumed that growth is retarded or halted during the formation of a transverse line, and that the density and width of the lines are related to the duration and severity of the illness or other incident that was responsible for the growth retardation. The common designation of these lines as "lines of arrested growth" also points to the presumption that interrupted growth is a prerequisite of their genesis. This assumption is supported by their prevalence in conditions which are often associated with growth failure such as severe and prolonged illnesses (1, 2, 3), severe nutritional deficiencies (4), and malnutrition (1, 5). Similar lines have also been found in a large percentage of children subjected to irradiation and other injuries incurred at the time of the atomic bombing of Hiroshima and Nagasaki (4). Furthermore they have been noted following prolonged treatment of leukemia with aminopterin (6). However, transverse lines have also been found in apparently healthy children (7), and it has repeatedly been observed that severe illness may fail to produce lines of increased density in one child, whereas the same illness of similar severity may produce a distinct line in another child of comparable age and sex (4).

The accidental finding that actinomycin D treatment in patients with Wilms' tumor was followed by the regular occurrence of transverse lines, offered an opportunity to study the formation of these lines in different children in response to a similar and reproducible causative agent. It has also been possible to study the significance of the development of multiple lines for the linear growth of the children.

MATERIAL

Between 1964 and 1968, 10 children were treated for Wilms' tumor at the Children's Hospital in Bergen. There were 7 girls and 3 boys and their ages ranged from [illegible] months to 5 ½ years. During this period, our therapeutic regimen consisted of nephrectomy as soon as the diagnosis of Wilms' tumor was established, followed by radiation in a dose of 3000 to 3600. Actinomycin D treatment was started in conjunction with surgery and continued through the next four days. The drug was administered intravenously in a daily dose of 15 μg per kilogram body weight. Following this initial treatment, a new course of actinomycin D therapy was given at intervals of two months for two years. In each course the patient received an intravenous dose of 15 μg per kilogram body weight per day for 5 days.

Two patients presented initially with metastases in the lungs. They died 2 and 4 months respectively after nephrectomy. Five patients have completed actinomycin D therapy and three are still under treatment. All these 8 children are alive and show no signs of recurrence.

OBSERVATIONS

During routine X-ray examination of the skeleton for the purpose of detecting metastases it was observed that each course of actinomycin D treatment resulted in the formation of a distinct transverse line of increased density. Characteristically the lines were located symmetrically in the skeleton (Figs. 1–3). They occurred most frequently in the distal end of the femora, both ends of the tibias and the distal end of the radii. Less frequently they were found in the proximal end of the femora and in both ends of the fibulas. Occasionally similar lines were found in the proximal end of the radii, the humeri and the first metatarsals. Analogous lines appeared frequently at the crest of the ileum, and in one child they were observed near the upper and lower edges of the lumbar vertebral bodies. The regularity with which the lines appeared was especially striking at the distal end of the femora where each course



Fig. 1. Knees of 2 ½-year old girl examined 2 months after the 4th course of actinomycin D therapy. There are 4 transverse lines.

of therapy was followed by a new transverse line in all the patients studied. In each metaphysis the lines were parallel to the contour of the epiphyseal end-plate and extended from cortex to cortex (Figs. 1–3). They were best developed at the knees and tended to be thickest and most dense in the youngest patients. The distance between the individual lines varied from child to child and in the different metaphyses, being greatest in the distal end of the femora and in the youngest child. As growth proceeded the lines became located in the diaphysis, underwent fragmentation, and ultimately faded away. This process of disintegration was most rapid at the distal end of the femora and in the youngest child. In this child, the first course of actinomycin D

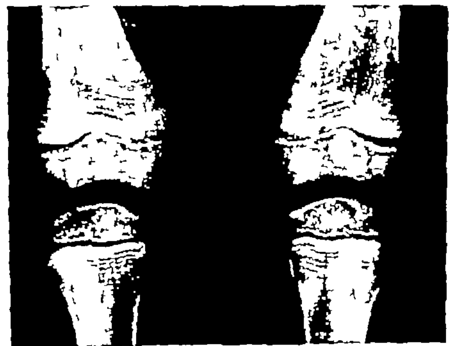


Fig. 2. Knees from same patient as in Fig. 1 examined months after 9th course of actinomycin D therapy. There are 9 transverse lines.



Fig. 3. Hips of 14 girl examined ... 2nd course of ... therapy. Note ... transverse lines and their parallelism to the epiphyseal end-plate.

treatment was given at the age of 8 months and the corresponding transverse line was buried in the shaft of the femur 6 months later. In another child who had started actinomycin D at the age of 2 years, a complete set of 12 transverse lines was counted at the knees at the end of the 2-year treatment period. However, in a somewhat older child starting the treatment at 5 years of age, the first three transverse lines at the distal end of the femora had disappeared upon completion of therapy 2 years later.

During the period of treatment, the linear growth of the children was followed by height measurements every second month. It was found that growth proceeded at the expected rate in all the children studied. The height data of four of the girls who had completed the actinomycin D treatment have been plotted on the growth chart in Fig. 4. It will be noted that the growth of each child proceeded in its individual channel and there was no "catch up growth" following cessation of therapy.

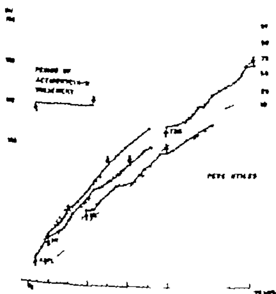


Fig. 4. Height data of 4 girls who had completed treatment plotted on growth chart. Growth proceeded at the expected rate.

DISCUSSION

The pathogenetic mechanisms underlying the development of transverse lines have been studied in rats by Parks and his co-workers and reported extensively by Parks (8). To obtain the desired growth arrest the rats were placed on a special diet which resulted in an almost complete cessation of linear growth in four to five weeks. The epiphyseal cartilage was then reduced to a thin plate acting as a barrier for the osteoblasts. However, osteoblastic activity continued on the horizontally disposed template produced by the under surface of the epiphyseal cartilage. The resulting bone plate, or primary bone stratum, was too thin to cast a radiographic shadow. With recovery from the condition inhibiting cartilage growth, the osteoblastic activity was restored and produced a distinct widening of the primary bone stratum which might be demonstrated radiographically as a transverse line of increased density. There are thus two factors in the development of the transverse lines. The first is the growth arrest which

is a sine qua non for the formation of the lines, and the second, the recovery factor which is responsible for their thickness.

The importance of the growth, or recovery factor in producing the transverse lines is reflected by their tendency to occur at the fastest growing bone ends. The fact that they were best developed at the distal end of the femora, and in the youngest child, also points to the significance of a high growth potential for their formation. Because the formation of each line could be precisely dated, it was also possible to obtain information on the process of disintegration of the lines in the different children and in the various metaphyses. The observations indicate that the growth factor is of significance both for the formation and disintegration of the lines, since it was found that their duration tended to be shortest in the fastest growing bone end in the fastest growing child.

The findings in this study make it quite evident that the growth arrest necessary for the occurrence of the lines may be of very short duration. It is also quite evident that an arrest of longitudinal growth is no prerequisite for their development, and that even the formation of multiple lines within a relatively short period does not ...ssarily imply any retardation of linear growth. The designations growth arrest lines or "lines of arrested growth should therefore be omitted since these terms generally convey an idea of arrested linear growth.

Although no direct proof was offered, it has been suggested that the transverse lines might result from a partial or complete cessation of growth hormone by the anterior pituitary (9) In recent years the mechanism of the action of hormones has been traced back to an effect on the regulation of DNA-directed m-RNA synthesis, and thereby to the regulation of enzyme production. Actinomycin D functions by specifically inhibiting DNA-directed m-RNA formation by binding to the guanine residue of DNA. This effect of the drug has been widely used in experiments designed to elucidate the action of hormones at the subcellular level (10). In such experiments it has also been demonstrated that actinomycin D is capable of blocking the basic action of growth hormone (11) Conceivably the drug may interfere with growth hormone-dependent chondrogenesis in the epiphyseal cartilage, and thus induce a transitory growth arrest sufficient for the formation of the primary bone stratum. The formation of transverse lines of increased density in response to actinomycin D treatment may therefore offer some circumstantial evidence for the assumption that temporary interruption of growth hormone secretion, or a transient block of the peripheral action of growth hormone, may be involved in the formation of these lines.

SUMMARY

Actinomycin D in a dose of 15 μg per kilogram body weight per day for 5 days was given at intervals of two months for two years in the treatment of Wilms tumor It was observed that each course of actinomycin D therapy resulted in the occurrence of transverse lines of increased density at the terminal segments of growing bones. Linear growth proceeded at the expected rate in all children studied. Evidently arrest of longitudinal growth is no prerequisite for the development of transverse lines, and even the formation of multiple lines within a relatively short period does not necessarily imply any retardation of linear growth. The formation of transverse lines in response to actinomycin D administration may indicate that interference with growth hormone secretion and/or peripheral action may be involved in the development of these lines.

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EVALUATION OF PROGNOSIS AND TREATMENT IN BELL'S PALSY IN CHILDREN

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Peripheral facial paralysis was recognised by Charles Bell in 1821 and the term "Bell's palsy" is now generally used for a facial paralysis of peripheral type and of an acute onset for which no local cause is found (13) It results from a unilateral intrinsic lesion of the seventh cranial nerve A viral etiology has been postulated but this can be excluded in most cases.

Bell's palsy is an important cause of morbidity in children as evidenced by the fact that it constituted 0 8% of all medical outpatients of the pediatric Dept. of Cairo University between November 1965 and October 1966. Being an uncomfortable and terrifying disease both to the child and his parents this incidence needs special attention both for prognosis and treatment. As there is a fair chance for spontaneous cure (7), a proper assessment of the prognosis is needed to avoid unnecessary and harmful interference.

In 187? Duchenne first described electrodiagnosis of facial palsies. Surprisingly it was not until Campbell (1954) (4) that nerve excitability tests have again been advocated to distinguish between physiological block and nerve degeneration. This was later confirmed by other works (5 8) Electromyography has been claimed to give reliable prognosis (8, ?6). However this method has the disadvantage that it is based on the detection of fibrillation potentials which appear late, up to 18 days after denervation and are seldom detected before 10 days.

There is no agreement about the treatment although many methods have been tried At present electrical stimulation of the paralysed muscles is widely used at least until voluntary movement appears. This practice is supported by many of the text books (3 ?8, 29), but Walshe (?7) states that galvanism is useless. Shambough (20) stresses the early treatment by vasodilators. Stellate ganglion block (23) and cortisone (25) have also been employed with favourable results, but the spontaneous improvement that so often occurs in Bell's palsy makes the evaluation of therapy difficult.

There is apparently no record in the literature which combines assessment of the degree of nerve injury as measured by nerve-excitability test together with the curative effect of different schemes of therapy based on this measurement. In the present study the aim was to fulfil these points in a controlled group of children with Bell's palsy It was felt justifiable to withhold electric therapy from the control patients as previous experience has shown that lack of electric therapy does not necessarily prejudice recovery from peripheral nerve injuries (11)

MATERIAL AND METHODS

The present study has been conducted on 150 patients (82 males and 68 females) referred from the outpatient clinic of the Pediatric Dept during the period from November 1965 to October 1966. The age ranged from 3–12 years 20 normal children of the same age range were also examined for the normal difference in nerve excitability between the two sides. Only those patients with a history of sudden onset of less than one week duration were included in the study Those having an upper motor neuron or associated lower motor neuron lesions were excluded. Thorough clinical, dental and aural examination were carried for every patient together with full history of the onset and predisposition of the disease if any Roentgenogram of the mastoid region was done when necessary to exclude mastoiditis. Electric examination of the facial nerve excitability was carried out as follows

A small cathode was applied to a point below the mastoid process at the exit of the facial nerve, like

Table 1 *Variations in nerve-excitability and degree of disfigurement at the onset of the d[illegible] d[illegible]fferent groups*

Group	Nerve excitability	Cases		Sex		Drooping of th[illegible] Severe		[illegible]
		No.	%	Male	Female	No.		
I	Unimpaired	123	82	68	55	89	73	
II	Diminished by less than 50 %	21	14	12	9	16	76	
III	Diminished by more than 50 % or absent	6	4	2	4	4	66	
Total		150	100	82	68	109	3[illegible]	[illegible]

[illegible] anode was placed at the back of the neck. The small cathode [illegible] as used to avoid direct stimulation of the facial muscles. The normal side was tested first and the minimal effective current intensity was noted while moving the cathode till the site of greatest response. The equivalent point on the affected side was then stimulated and the intensity of the current to produce contraction was recorded. Forty-two patients (26.7%) were tested on the 3rd day of the disease, 65 patients (43.3%) on the 4th and 5th days and 43 patients (26.7%) on the 6th and 7th days.

The patients were grouped according to the results of nerve excitability measurement into the following groups.

Group I Unimpaired excitability indicative of physiological block [illegible]hen the affected side responds fully at the same current intensity as the normal. These patients were further subdivided into the following subgroups.

Group IA. Forty-one patients received no treatment except reassurance.

Group IB Forty-one patients received short wave therapy to the affected side for 15 minutes 3 times per week for 1[illegible] sittings or till recovery

Group IC Forty-one patients received nicotinic acid by mouth twice [illegible] day in [illegible] dose of 50 mg each or in sufficient dose to cause flushing.

In all patients in group I physiotherapy was given.

Group II Excitability diminished by more than [illegible] mA and less than 50[illegible] of the normal side [illegible]hich indicates partial nerve degeneration. These patients received after the first week of onset of the disease short wav[illegible] therapy to the affected side, followed by labile anodal galvanic stimulation together with physiotherapy

Group III Excitability diminished more than 50% of the normal side or was absent which indicates complete degeneration to the nerve.

The same schedule of treatment as group II [illegible]as given. The patients [illegible]ere followed up for one year during [illegible]hich they were examined weekly for the first month and then every two weeks till maximum clinical and electrical recovery occurred.

Recovery [illegible]as classified as:

1 Complete in [illegible]hich the difference in nerve excitability between the two sides [illegible]as not more than [illegible] mA [illegible]ith no distortion of the nasolabial fold or orbital fissure at rest or on active movement.

[illegible] Incomplete in [illegible]hich there is [illegible] diff[illegible]enc[illegible] in [illegible]citability between the t[illegible] sides [illegible] more than [illegible] some distortion on active movement

3 Bad response in [illegible]hich the difference in [illegible] [illegible] marked and distortion [illegible]as noticed at re[illegible] and active movement.

RESULTS

The results are presented in Tabl[illegible] 1 an[illegible] [illegible] d Fig. 1 The results of the nerve exc[illegible] [illegible] m[illegible] urements of the [illegible]0 normal childre[illegible] [illegible] [illegible] that the maximum difference between the two [illegible]d was 2 mA.

DISCUSSION

The results of the present investigation stress the value of early evaluation of nerve-excitability in the prognosis of Bell's palsy The cases showing persistant unimpaired excitability during and after 7[illegible] hours gave 100[illegible] cure, all that they needed was reassurance vasodilators and training for active exercises of the facial muscles to cover the period of recovery Recovery rate ranged from 1–6 weeks. It is evident that the nerve in these cases suffered from physiological block and that the minimal time of recovery in this group (one week) correspond to the time of resolution of inflammatory and vascular reactions which caused compression of the nerve within the facial canal. In the group of patients who received short wave therapy (group I B) recovery was achieved from [illegible] to 6 weeks and the incidence was 100%

This shows that short wa[illegible]e therapy does not help rapid recovery in cases of physiological block. Henderson & Taverner's experience (26) shows that lack of electrotherapy did not pre

Table 2. *Schedule and outcome of treatment in different groups*

Group	Treatment: Physiotherapy	Nicotinic acid	Short wave	Galvanism	Maximum recovery: Rate	Complete	Incomplete: Distortion on movement	Distortion at rest and movement
I A	+	–	–	–	1–6 W	41 (100 %)	–	–
I B	+	–	+	–	2–6 W	41 (100 %)	–	–
I C	+	+	–	–	1 5 W	41 (100 %)	–	–
II	+	–	+	+	10 W–4 M	19 (90 %)	2 (10 %)	–
III	+	–	+	+	3–6 M	–	4 (67 %)	2 (33 %)

judice recovery from peripheral nerve injury Mosforth & Taverner (15) treated half their patients by infra-red radiation followed by galvanism and did not notice significant difference between the two groups. The fact that the shortest period of recovery in our cases (group I B receiving early short wave) was nearly double the corresponding period of group I A (receiving no treatment) gives an impression that early short wave therapy may cause delay in the onset of recovery This delay could be the result of increased pressure from vascular congestion within the facial canal, on an already swollen nerve.

In favour of this conclusion is the description by Cawthorne (6) on information gained from direct exposure and inspection of the nerve. He described the nerve as being unduly constricted at the level of the stylomastoid foramen with marked edema and swelling of the nerve in its bony canal. Kettel's observation (14) during decompression operation was similar to that of Cawthorne It is believed that an ischemia of the nerve due to arteriolar constriction initiates a vicious circle, ischemic damage resulting in venous congestion and edema (12, 14). These in turn, would compress the nerve in the rigid Fallopian canal causing an accentuation and perpetuation of the ischemia. The initial vasospastic ischemia follows an abnormal cooling of the region around the stylomastoid foramen in about 70 per cent of cases. In the remainder severe emotional upset or shock has been noted. James & Russel (13) concluded that when Bell's palsy develops, the nerve is being blocked by increasing pressure within the facial canal which is secondary to some inflamatory or vascular reaction in the neighbourhood, and that the pain which often precedes the acute stage is presumably due to this same reaction.

In group II where nerve excitability was impaired more than 2 mA (which was found to be the maximum variation between the two sides in the normal controls) and less than 50% than the normal side (partial degeneration of the nerve) incidence of recovery was 90%. The minimal time for maximal recovery was 10 weeks, which is the time compatible with 1 inch/month as the functional rate of regrowth of the nerve (5)

Treatment of this group was devised to preserve the viability of the muscles during the period of recovery by interrupted galvanic stimulation 3 times per week together with daily exercises of the facial muscles. Campbell *et al* (5) advises daily galvanic stimulation of muscles, while Mosforth & Taverner (15) did not find marked difference between treated and untreated groups. In our series no case developed contractures and it was felt that galvanic stimulation

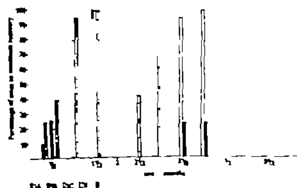


Fig. 1

3 times a week was valuable for maintaining the viability of the muscles and boosting the morale of the patient and his parents, in the longer anticipated convalescence than patients with physiological block. In the third group in which excitability was diminished by more than 50% or absent after the third day (complete degeneration of the nerve) a poor prognosis was expected. Only 66% recovered with mild drooping of the facial muscles while 33% were left with marked disfigurement of the face. This group received the same schedule of treatment as group II

It is interesting to note that the incidence of physiological block in children with Bell's palsy was found to be 82% in comparison with 55% in Campbell's adult series and 50% of James & Russell's adult patients. This may be due to the fact that the facial canal in children is more yielding than in adults. In the newborn, the facial canal is not yet bridged over by bone (20) Whatever the mechanism, venous congestion and edema in the bony canal are believed to be the most constant pathologic features of Bell's palsy The physiologic effect of short wave diathermy is due to the rapidly alternating current of high voltage each phase of such current is shorter than the excitation time producing rapid oscillation of ion that causes heating of the tissues without stimulation (24). This deep seated heat results in hyperaemia and vasodilatation of blood and lymph vessels, and this depends on the intensity of the current, size of electrode and resistance of tissues (19). It is thus evident that short wave diathermy which is beneficial in acute inflamatory processes in other parts of the body may not be the favourable therapy specially in the early stages, in cavities with limited capacity as in the rigid Fallopian canal. The local vasodilatation of all the blood vessels (arterioles, capillaries and veins) and lymph vessels, the degree of which depends in part on the resistance of tissues may increase the pressure within this rigid canal on an already swollen nerve.

In group I C which received nicotinic acid, the percentage of cases recovered at 1 week (25%) was more than double those of group I A which received no treatment (10%) (see the chart). The same was noticed at 2 weeks where cases recovered in group I C were double those of group I B and much more that those of group I A. Recovery was complete in 100% of the cases at an earlier time than the other two gr p 5 weeks). Comparison of the results stres th beneficial effect of systemic vasodilators l short wave therapy Nicotinic acid c he vicious circle of arteriolar spas iates ischemic damage and venous cc iting in a better blood flow without m pr ure (2) while short wave causes dila blood vessels and lymph vessel gestion and pressure on the ner (21) stresses the value of vasodilators he stages of the disease and advises n in ambulatory patients. Perri (16) g results from intravenous histamine in sa by drip at a rate that causes flush severe headache, while Skinner (2) reported go results from subcutaneous injection and s ol n drops. Stellate ganglion block has also been with favourable results (23). For children w Bell's palsy we found nicotinic acid to b the most suitable vasodilator from th point of view of administration and side effects

It was also noted that the severity of facial muscles affection in children was not in p por tion to the nerve injury In many cases o gr ups I and II in which nerve excitability was minimal or not impaired, drooping of the paralyse si of the face was marked. Mildly affected cases ha the same incidence in the three groups Gowers (10) considered that this drooping depends largely on the age of the patient. James & Russell (13) stated that the important factor is the patient's habits as regards facial expression. Over used muscles sag most when paralysed. It seems that the younger the age of the patient, the more actively the muscles of face are used before paralyses sets in and the more they sag when paralysed regardless of the degree of the nerve injury

SUMMARY

Nerve-excitability measurements performed early in the course of the disease are of a good prognostic value. The results show that when excitability is maintained unimpaired after 72 hours of the onset, there is a chance of 100% spontaneous cure. When the excitability is diminished by less than 50% 90% recovery may occur but when it is markedly diminished or absent the prognosis is bad.

The schedule of treatment to be followed should

be based on the result of nerve excitability measurement early in the course of the disease.

Systemic vasodilators, as nicotinic acid early in the course of the disease has excellent effect as it cuts the vicious circle of arteriolar spasm without increasing pressure within the facial canal.

It is advisable that early short wave therapy should be avoided as it may increase nerve compression through local vascular congestion in the vicinity of the nerve.

Incidence of physiological block is higher in children with Bell's palsy than in corresponding adult patients who, as a result have a higher incidence of nerve degeneration and worse prognosis.

The severity of paralysis does not indicate the degree of nerve lesion.

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CRYPTORCHIDISM

Roentgenological and Surgical Aspects

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For centuries cryptorchidism has been known to impair fertility It is a fairly common abnormality and in recent years it has given rise to much discussion regarding proper modes of therapy optimum age for undertaking treatment and the result that can be expected from therapy

In 1966 and 1967 Lunderquist & Rafstedt (21 22) reported a new roentgen method, andrography for demonstrating undescended testes and the present paper is concerned with the results obtained with this method. The method demonstrates not only the anatomic conditions in cryptorchidism but often also their cause.

The diagnostic reliability of the method is compared with that of palpation and with surgical findings, and indications for the use of the method are given.

The optimum age for operation is also discussed.

Definitions differ from author to author We consider the following definitions most suitable from the roentgenologic point of view

Retentio testis designates a testis retained somewhere along its normal path of descent w having passed through the inguinal canal

Ectopia testis a testis that has passed thro g the inguinal canal but then deviated from h normal path to occupy an abnormal position

Pseudocryptor hidism retracted te tis or mob le testis, is a normally descended testis that has been drawn up to or into the inguinal canal b ne re master (here are also included those nst nces newborns in whom the descent is not yet complete)

METHOD AND MATERIAL

With an ordinary injection needle the left iliac fossa is punctured at point 5 cm from the left anterior superior spine of the ilium on line between the latter and the umbilicus, i.e., at site corresponding to McBurney' point N$_2$O is then injected intraperitoneally 20–30 ml in newborns and 600–800 ml in 10–15 years old children Films are taken with the patient prone in the head-down position (40–45°). The radiation to each newborn is exposed is about 5 mr per film.

The newborns are examined in the maternity ward,

Table 1 *Distribution of all patients examined with andrography*

Age (years)	Number of patients investigated	Number of testes investigated			Andrographic diagnosis: Pseudocryptorchidism			Retentio testis			Ectopia testis		
		Dxt.	Sin.	Bilat.	Dxt	Sin	Bila	Dx	Sin.	Bilat	Dxt.	Sin.	Bilat.
0–1	16	15	8	6	4	2	4	4	4		7	2	
1–5	4	3		6						4	3		
6–10	22	6	8	16				1	4	6	5	4	10
11–15	8	2	4					2	1			3	
Total	60	26	20	[illegible]	4	2	4	7	9	1	13	9	1

Table 2. *Comparisons between oentgen, palpatory and operative findings*

Number of testes

Age (years)	Number of patients operated upon	Number of testes operated upon: Dxt.	Sin.	Bilat.	Andrographic diagnosis: Retentio: Dxt.	Sin.	Bilat.	Ectopia: Dxt.	Sin.	Bilat.	Roentgenographic misdiagnosis: Retentio	Ectopia	Surgical diagnosis: Retentio: Dxt.	Sin.	Bilat.
0– 1	6	5	1		1			4	1				1		
1– 5	4	3		2			2	3			2				
6–10	21	5	10	1		5	4	5	5	8	4			4	
11–15	5	2	3		2	1			2		2		1		
Total	36	15	14	14	3	6	6	12	8	8	8	0	2	4	0

the other children in the outpatient department. No complications were noted.

The clinical material is summarized in Tables 1–4. Table 1 includes all the patients examined with andrography and Table 2 only those operated upon. The material also included 5 intersexes; these will be published separately

RESULTS

The frequency of cryptorchidism was assessed on the basis of the records of all children born at Ängelholm hospital during a 3-year period (1964–1966). Of the 2229 full-term boys born alive dur that period, 23 had cryptorchidism. Of these 7 proved to have pseudocryptorchidism, i.e., the testis descended spontaneously within a few months. After exclusion of these cases the frequency of cryptorchidism was 0 7 %

Of the 60 cases of cryptorchidism (Table 1), 26 (43 %) were right-sided, 20 (33 %) were left-sided and 14 (23 %) were bilateral.

It is clear from the Table 2 that ectopic testis occurred in 30 (83 %) of 36 cases and further that the roentgent diagnosis was correct in all of the patients in the 0–1 year age group.

The primary results of operation in the 0–1 and 1–5 year groups were invariably good (Table 3). In no instance was the operation technically dif ficult.

Operation also revealed that 4 of the retained testes and 10 of the ectopic testes were macroscopically atrophic.

Biopsy specimens of 7 testes were obtained and examined histologically The results are given in Table 4

Of the cases operated upon because of ectopic testes, the operation revealed a large hernial sac in 22 and a small one in 10. In the remaining 5 as in all the cases with retained testes, there was no hernial sac.

DISCUSSION

Roentgen diagnosis. When gas passes down through the inguinal canal and fills the tunica vaginalis, an ectopic testis may be assumed even though the

Table 3 *Results of operation for retentio and ectopia testis*

Age (years)	Surgical results: Retentio: Good	Poor	Ectopia: Good	Poor
0– 1	1		5	
1– 5			5	
6–10		4	18	5
11 15	1		1	3
Total	2	4	29	8

Table 4 *Biopsy findings*

Age (years)	Biopsy: Retentio: Atrophy	Normal	Ectopia: Atrophy	Normal
0– 1	1			1
1 5			1	
6–10	1		2	
11 15				1
Total	2		9	2

		Physical diagnosis (palpation)						
…pia			Retentio			Ectopia		
…t	Sin.	Bilat.	Dxt.	Sin.	Bilat.	Dxt.	Sin.	Bilat.
	1		1	1		4		
		2	1			2		2
	6	12	1	5	2	6	5	8
	3			1		2	2	
	10	14	3	7	2	14	7	10

…ended testis can not be demonstrated. In such cases the tunica vaginalis is nearly always displaced laterally (Fig. 1) If a filling is obtained (in newborns) of a large tunica vaginalis that is not displaced, it is a sign of pseudocryptorchidism (Fig. 2).

A small tunica vaginalis adherent to the testis, will not be filled with gas. This may be misinterpreted as a sign of a retained testis (Fig. 3).

An intraabdominal testis is not roentgenographically demonstrable because it is situated between the peritoneum and the posterior abdominal wall. Only in one case (mixed gonadal dysgenesis) could such a testis be demonstrated, but it was misinterpreted as an ovary because it occupied the normal position of the ovary and because the patient had an uterus.

Frequency Earlier surveys of undescended testes before puberty produced figures varying from 3.3% to 9.4% (22, 28). Ward & Hunter (33) reported only 2.5% while The East Anglian Branch of the Society of Medical Officers of Health (32) gave as high a figure as 6.1% Scorer (25) and Cour Palais (9) reported cryptorchidism with a frequency of 0.7% and 0.78% respectively in children above 1 year

Unilateral cryptorchidism is more common on the right side than on the left. In Scorer's (26) series the condition was right-sided in 48% left sided in 41% and bilateral in 11% The corresponding figures reported by Gross *et al.* (12) were 45% 30% and 25% which are practically the same as those found i the present material.

Gross *et al.* (12) claim that cryptorchidism is associated with an indirect inguinal hernia in as many as 90% of all cases, while Lauritzen (20) gives a lower frequency namely 50–70% a figure corresponding to that noted in our material.

Both Gross *et al.* (12) and Lauritzen (20) belie





Fig. 1 Age 11 years. Right-sided cryptorchidism. Right testis ectopic situated laterally to the external ring of the inguinal canal and in gas-filled tunica vaginalis hich extends obliquely upwards. The upper pole of the testis is fixed by adhesions. Verified by operation.

Key for tracing Adh. Adhesions, B, bladder G gubernaculum testis, P pubic bone T testis; TV tunica vaginalis, F thigh bone



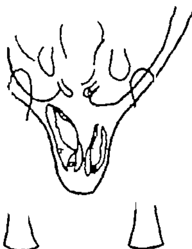


Fig. 2. Age 2 days. Right-sided pseudocryptorchidism. Gas passes down the inguinal canals and fills both tunica vaginalis, which extend down to floor of scrotum. The otherwise typical traction of tunica vaginalis obliquely upwards is missing. Left testis is in the scrotum, the right one at the external ring of the inguinal canal. Both testes are fastened to floor of scrotum with gubernaculum testis. Re-examination 2 months later showed both testes descended.

cryptorchidism to be due to mechanical factors. According to Lauritzen ectopia is due to a defect of the anlage or growth of the gubernaculum testis so that after the testis has passed the inguinal canal it is deflected upwards and laterally. This was corroborated by the present investigation. In pseudocryptorchidism the gubernaculum testis was seen in its normal position (Fig. 2), while in ectopic testis it was not demonstrable. Those adhesions which in ectopic testis fixed the testis upwards and laterally may have been due to defectively inserted gubernaculum testis (Fig. 1).

At surgical investigation Gross and coworkers as well as Lauritzen found that the testis was adherent to the tunica vaginalis and that among the cremaster fibres were tight cords which prevented the descent of the testis. These adhesions have been explained embryologically by Backhouse (2) and they can often be demonstrated by andrography (Fig. 1).

Fertility. It is generally believed that the relatively higher temperature in the abdomen is the cause of injury to the undescended testis. The difference between the intraabdominal and the intrascrotal temperature is said to be 2.2°C, while the difference between that of the scrotum and that of the inguinal canal is 0.9 C (17).

It is widely agreed that bilateral cryptorchidism results in infertility but it is often stated that fertility is normal as long as one testis has descended. Bergstrand & Qvist (3) found that in patients operated on for bilateral cryptorchidism the number of fertile men seems to be between 35 and 55%. Scott (27), however found that 70% of men with an undescended testis were either infertile or subfertile, and Hansen (14) showed that the quantity of semen produced and the sperm count in such cases were about half the average normal. In similar cases Engberg (11) found a reduction of the excretion of androgen in the urine.

Though it is generally accepted that the undescended testis is injured, opinions differ regarding the elapsed time required to produce such

injury While Cooper (8) observed testicular degeneration in -3 years old children, Hinman (15) and Alojzy (1) believe that such injury does not occur below the age of 6 and Snlffen (31) Robinson & Engle (24), and Charney & Wolgin (6) that they appear at 8–10 years. Bergstrand & Qvist (4) believe that no grossly visible and evident atrophy arise before the age of 10–11 years. Hansen (14) is of the opinion that they do not occur until puberty

Charney (7) demonstrated that the lesions are irreversible.

In the present material 4 out of 6 retained testes were macroscopically athropic, against 10 out of 37 ectopic testes. Microscopic signs of atrophy were seen already in the 0–1 year age group (Table 4).

Therapy No unanimity has been achieved concerning the age to which treatment may be postponed. While Ward & Hunter (33) believe that 70 ,» or more will descend at puberty Williams (34) stated that "most surgeons of long experience have voiced the opinion that spontaneous descent is rare and Scorer (26) like Cour Palais (9) is of the opinion that testes not descended by the age of 1 year will seldom, if ever descend.

On the basis of the present material it appears that those testes which descend spontaneously after the age of 6 month were in reality retracted testes, which with proper palpation should be distinguishable from undescended testes. At any rate spontaneous descent after the age of 1 year is too rare to warrant watchful waiting, which might result in irreversible injury (7).

While Smith (29) claims that 2 years is the most suitable age for operation Gross *et al* (13) believe the optimum age to be 9–11 years. According to Koop (19), the most suitable ge is 1–2 years if treatment is to include operation on a coexisting hernia.

Our material seems to corroborate the opinion that 1 year is a suitable age for operation, but only of ectopic testis, which, however constitute about 83% of all cases. We were able to confirm the finding of Gross *et al* (13) and of Lauritzen (20) that by this age the testis has a ufficient length of spermatic cord, which makes the operation technically easy Loosening of the adhesions is often sufficient to allow the testis to occupy its proper place in the scrotum. In our series the roentgenological diagnosis in the 0–1 year group



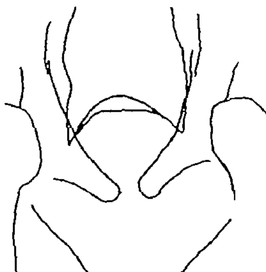


Fig 3 Age 6 years Bilateral cryptorchidism. No gas passes down through the inguinal canal N filling obtained of the tunica vaginalis. Retentio testes. Verified by operation.

was invariably correct. Thus, should palpation not allow a firm diagnosis andrography is indicated.

The primary results of operation were always good (Table 3)

A for retentio testis, we prefer to give expectant treatment because the operation seems to carry a considerable risk of vascular injury with testicular atrophy as a result.

Andrography has proved very useful in the early diagnosis of intersex. In all 5 cases examined by us we could demonstrate either undescended testes or internal female genitals. The examination can be performed with advantage already during the neonatal period.

On the basis of the above investigation it is concluded that andrography is indicated in the investigation of cases of *cryptorchidism* when the testes can not be palpated and in *intersexes*.

The examination should preferably be performed within the first 6 months of life

SUMMARY

60 cases of cryptorchidism and 5 intersexes were examined with andrography (pneumo-pelvigraphy) Of these, 36 were operated upon.

The roentgen findings are compared with the palpatory and surgical findings. The value of the method is discussed and the indications for it are given.

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THE INFLUENCE OF HORMONE TREATMENT ON THE NATU EVOLUTION OF THE IDIOPATHIC NEPHROTIC SYNDROME IN CHILDHOOD

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There is no doubt that the evolution of the idiopathic nephrotic syndrome (INS) has been thoroughly influenced by ACTH and steroid treatment. The beneficial immediate effect of this treatment is beyond doubt. The long-term effect, however is still a matter of discussion. the initial optimistic reviews were not always confirmed by subsequent follow-up studies. The aim of the present study was to contribute to the solution of the problem by comparing the evolution in the pre hormonal and in the hormonal era in patients with a sufficiently long follow-up period. The results obtained in the pre-hormonal group, comprising 44 patients, were published in 1966 (15). The present paper deals with the results obtained in a similar group from the hormonal period, and also with the results of a comparative study of both groups.

MATERIAL AND METHODS

In this department, hormone treatment of the INS was started in 1952. In order to avoid as much as possible selection of the material, the first 44 patients with INS admitted since 1952, were studied. This represents an equally large group as was studied in the pre-hormonal period

The criteria on which the diagnosis of INS was based, ere the same as for the pre-hormonal group, and ere the generally accepted ones: edema, marked proteinuria, hypoalbuminemia and hyperlipemia. Transient hematuria, hypertension or renal insufficiency were considered to be compatible with the diagnosis of INS. Renal biopsies were not yet performed ystematically at onset of the disease in this group and therefore histologic criteria were not taken into consideration. Secondary NS and two patients th congenital NS ere excluded from this study

The age- and sex-incidence is represented in Fig 1 The average age at onset of the INS was 5 years, with p incidence between 2 and 3 years. One child was y than 12 months, and 4 were older than 10 years. There were 33 boys and 11 girls. From this group of 44 patie one girl who lived abroad, was excluded later from th study as the final control investigation could not be pe formed. Forthy-three patients were thus left f study

In the course of the years personal experience and li erature data have prompted us to adapt our treatment program. Four different periods can be distingu shed, is illustrated by Table 1

The study of the evolution of the INS in these 4 patients is based on the results of frequent investigations du ing the acute illness, of subsequent control-investigations and of late re-investigation. The investigations alw ys consisted of a complete clinical examination with repeated measuring of the blood pressure, of urinalysis and serologic determinations. Mostly and without exception during the late re-investigation, glomerular function as also measured. In some patients kidney functions were studied more extensively In some, renal biopsy was also performed during control investigation. The resulting histologic data were taken into account for evaluation of the present condition of the patient. With the help of these data, the evolution was re-constructed in the 43 patients over period ranging from 6 eeks to 14 / years. The follow-up periods in the surviving 40 patients vary from 4 years to 14 / years as is represented in Fig –

The definitions of the different evolutionary stages observed in these patients were the same as those used in the pre-hormonal group The definition of remission, stage hich was not seen in the pre-hormonal group, as added. The NS as considered to be active as long as the typical clinical, serologic and urinary abnormalities were observed. The evolution to renal insufficiency was seen as continuation of the active process. Serologic normalisation was accepted when total proteins, electrophoretic pattern and lipemia ere normal. The absence of proteinuria or the finding of less than 1 g of proteins in the urine of 24 hours, was taken as criterion of urinary normalisation When serologic and urinary normalisation as observed, hen blood pressure and kidney

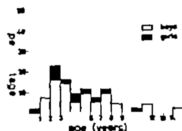


Fig. 1. Sex and age incidence in the 43 hormonally treated patients.

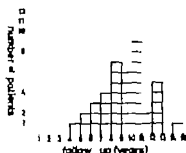


Fig. 2. Diagram showing the length of the follow-up period in each of the 40 surviving patients from the hormonal group.

tests were normal, and when eventual histologic examination of kidney biopsy disclosed no active lesions, the disease was considered to be either healed or in remission. When all these criteria were fulfilled since at least 3 years in a patient who had not been treated since at least 2 years, this was accepted as complete healing. When this had occurred since less than 3 years, or when treatment had been given in the last 2 years, this was considered as remission. We are aware of the arbitrariness of this distinction as the occurrence of a relapse always remains a possibility. Late relapses after unusually long periods of remission were classified separately. Hypertension and solitary proteinuria of more than 1 g in 24 hours in a patient who was otherwise completely normal, were defined as sequelae.

RESULTS

The reconstruction of the evolution in the 43 patients is reproduced in Fig. 3. From this, it appears that, after a variable period of activity the INS healed in 67.5% of the patients, that it was interrupted by death in 7% and that in two patients (4.6%) a longstanding remission was followed by a relapse. In one patient sequelae were observed. Finally in 18.6% the evolution of the INS was characterized by multiple recurrences, alternating with more or less longstanding remissions.

Closer examination of each of these groups reveals other particularities. In the first place, two smaller groups can be distinguished among the cured patients. In the first subgroup comprising 8 children, the INS ran a relatively short course of 6 weeks to 5 months, and never relapsed afterwards. In one patient (11) healing was so fast that not treatment was needed. These acute nephroses were also observed in the pre-hormonal era. In the other 21 patients definitive healing occurred after a period of activity of more than 6 months, eventually interrupted by periods of remission. On the average, the INS had an activity of 20 months in this subgroup, before definitive healing could be accepted. In 7 of these children this was due to the slow urinary and serologic normalisation, without one single relapse occurring. In the majority of the 21 patients, namely in 14 of them, it was due to the occurrence of one or more

Table 1. *Different treatment programs applied in the patients under discussion*

1952–1954	One 10-day course of ACTH (75–100 mg/d)	*No maintenance therapy*
1955–1957	Initial 10-day course of ACTH (75–100 mg/d)	*Maintenance therapy*: ACTH (75–100 mg/d) on 3 consecutive days followed by 11 days without treatment until 2 months after complete serologic and urinary normalisation *if*: illness of more 3 months duration, relapse, no improvement after initial course
		No maintenance therapy if improvement after initial course
1957–1959	Initial 10-day course of ACTH (75–100 mg/d)	*Maintenance therapy*: Same as 1955–1957 *but* after 12 months of illness ACTH replaced by Prednisone (40–60 mg/d) on 4 consecutive days/week, until 4 months after complete normalisation
		No maintenance therapy if improvement after initial course
1959–	Initial 10-day course of Prednisone (40–60 mg/d)	*Maintenance therapy*: Prednisone (40–60 mg/d) until 3 months after complete normalisation

relapses. In all, 20 relapses were noted, i.e. 1.5 a patient. No patient had more than relapses. The remissions lasted from 1 to 27 months. When the whole group of the 21 non-acutely cured patients is considered, this represents somewhat less than 1 relapse a patient. Histologic data were available in 13 cured patients. Kidney biopsies were performed 11 months to $10\,^{8}/_{12}$ years after onset of the NS, with an average of $5\,^{7}/_{12}$ years. In 11 patients no abnormalities were seen. In 1 patient (I.) a small focus of interstitial cellular infiltration and fibrosis was seen. Urinary infection was absent. In another patient (I9) $^{1}/_{8}$ of the glomeruli showed hyalinisation and sclerosis. Small foci of tubular atrophy and lymphocytic infiltration were also present. No active glomerular or tubular lesions were seen. As in the other cured patients, kidney function tests yielded normal results (Inulin clearance 162 ml/min/1 73 m², PAH clearance 597 ml/min/1 73 m² concentrating capacity 1076). This patient presented a pronounced but transient tubular syndrome which was discussed elsewhere (1.).

Three patients died respectively 6 weeks, 5 years and $7\,^{1}/_{2}$ years after onset of the NS. Patient V3 died of coli-peritonitis, a few days after interruption of the first 10-day course of ACTH treatment. In patient V2 death occurred after thrombosis of the pulmonary artery a known but exceptional cause of death in nephrotic patients (7 1). Postmortem examination in this child, however revealed extensive glomerular fibrosis which undoubtedly would have led to death in the next years. No biochemical symptoms suggested renal insufficiency in this patient after an evolution of 5 years. In patient V1 renal clearance decreased and arterial hypertension was observed after an evolution of only 3 years. Progression of the renal insufficiency led to death 7 1/ years after onset of the disease. At autopsy complete hyalinisation of practically all glomeruli was found.

In two patients the INS relapsed after a normalisation of respectively more than 9 years and practically 4 years. I the first patient (III1) steroid treatment, which is still going on, resulted in a new remission. In the second patient (III?) this treatment resulted n a normalisation, which now lasts since 4 years. I this last patient the NS I probably cured as no treatment has been given since 3 years.



Fig. 3 Overall picture of the evolution in the 43 p ien ts from the hormonal period The obser ed cates ries o patients are indicated by roman numerals, the patien arab figures. For every patient the total length of h column represents the length of the follow-up period as indicated by the figures on the ordinate The dark and dotted columns represent the length of the periods of activity and remission respectively

In one patient (IV1) an isolated hypertension and a proteinuria were observed. After two relapses reacting well on steroid treatment, a hypertension occurred during the treatment of a third relapse. A slight proteinuria and disturbed renal concentrating capacity were also present, but all other kidney function tests remained normal. I uline clearance was 10. ml/min/1 73 m² PAH clearance 593 ml/min/1 73 m² PSP excretion 23% in 15 minutes. A kidney biopsy performed 10 years after onset of the NS showed no abnormalities. A second biopsy performed years later revealed hyaline deposits in the intima of the small arterioles and a hyperplasia of the media. A small focus of interstitial lymphocytic infiltration was also seen.

Because of the higher number of relapses and hence the longer period of activity 8 ı

Table 2. *Kidney function, blood pressure and kidney biopsy data in the 7 hormonally treated patients with multiple relapses*

Clearance values are expressed in ml/min/1 73 m². The figures in parentheses represent the time, in years and months, between the onset of the NS and the investigation in question

Patient	Clear inulin	Clear creatinine	Clear PAH	Concentrating capacity	Blood pressure	Renal histology
II1	80 (12.8)	—	498 (12.8)	—	150/100 (12.8)	Fibrosis and hyalinisation of some glomeruli Hypercellularity of other glomeruli Hyalinisation and substenosis of the vas affer Tubular atrophy Interstitial infiltration (12.8)
II2	146 (12.3)	151 (12.3)	765 (12.3)	1027 (12.3)	140/90 (12.3)	No abnormalities (12.3)
II3	159 (8.9)	179 (8.9)	586 (8.9)	1025 (8.9)	130/80 (9.9)	No abnormalities (9.9)
II4	180 (8.5)	155 (8.5)	603 (8.5)	1033 (8.1)	110/70 (8.5)	No abnormalities (2.7) * No abnormalities (8.1)
II5	—	104 (5.9)	631 (6.6)	1027 (6.1)	110/70 (6.1)	No abnormalities (1.8) * N glomeruli. Normal tubuli and interstitium (6.1)
II6	—	180 (6.7)	—	1029 (6.7)	100/60 (6.7)	No abnormalities (4.8)
II7	102 (6.11)	119 (6.7)	609 (6.11)	—	135/70 (7)	No abnormalities (6.7)

were classified separately. Within 6 to 12 years, 7 of these children had at least 3 but sometimes up to 13 relapses. The average number of relapses in this group is practically 6 a patient. As a consequence, the average duration of the NS calculated from onset of the disease to last normalisation, has increased to 8 years 5 months. The duration of the remissions is extremely variable: It varies from 1 month to several years. Only in patient II1 true steroid dependency is present: lowering of the steroid dosage below a certain level always resulted in a prompt relapse. Relapses, however also occurred in this patient during treatment with higher dosages. As will be discussed later the evolution in these patients is probably decisive for the interpretation of the long-term effect of hormonal treatment. Therefore the results of the last extensive control investigations are reproduced in Table 2. It appears from these figures that for the moment being, there are no indications for a fatal issue in 6 out of these 7 children. In patient II1 however kidney biopsy revealed obvious lesions. Some glomeruli showed complete fibrosis and hyalinisation. In the other glomeruli an unmistakable hypercellularity of the tuft was seen. Numerous glomeruli showed adhesions between parietal and visceral layer. Another important finding was the pronounced hyalinisation of the vas afferens leading to substenosis of the lumen. Areas of tubular atrophy and lymphocytic infiltration of the interstitium were also present. The clearance values were at the lower limit, as is indicated by Table 2. A moderate and transitory elevation of the blood pressure was observed in this patient but was attributed to the steroid treatment.

Patient II8 was also classified in this group, although he had only one relapse of his NS. Neglecting our recommendations, the parents treated him with usually low doses of steroids during more than 7 years. A serologic normalisation was obtained after 17 months, but a variable proteinuria remained. The possibility that this case will have to be classified later in the group of healed patients is certainly not illusive, although premature for the moment.

Fig 4 represents the comparison of the results obtained in the pre-hormonal and in the hormonal

group. Altogether there are 17.5% more cured patients in the hormonal period, whereas 34% less deaths are noted. The group with multiple relapses is observed only in the hormonal period. The number of late relapses and sequelae is nearly identical for both periods.

Comparison of the separate groups also permits some conclusions. The number of dead hormonally treated patients is too small to allow a valuable comparison. One has none the less the impression that, as opposed to the pre-hormonal era, evolution to death is not always obvious after a 4-year course in hormonally treated patients.

As for the cured patients, it can be stated that acute nephroses occur in both periods in an almost identical percentage (22.7% and 18.6% of all patients). An important difference, however characterizes the course of the INS in the slowly healing patients. Since hormone therapy was used, the average duration of the disease in these patients not only increased significantly from 11 months to 20 months, but this is mainly due to a higher number of relapses: an average of nearly 1 relapse a patient is seen as opposed to only relapses for the entire pre-hormonal era.

DISCUSSION

Both groups, which were studied here, are comparable enough to permit valuable conclusions. The groups comprise 44 and 43 patients, who were studied consecutively in order of their admission and without preliminary selection. In all patients the diagnosis of INS was based on the same criteria. All 87 patients were examined frequently in the same department by the same investigator (C. H.). Moreover sex and age incidence are practically superposable in both groups. A pre-selection of the material, resulting from the sending of mainly steroid-resistent cases to this department, did not yet come into play. As renal biopsies were not performed systematically histologic criteria were not considered. The homogeneity of the material makes it however probable that the different possible histologic varieties are equally spread over both groups.

The inevitable changes in the treatment programs, based on personal experience and literature data, limit the possibilities of this work to a general study of the influence of hormone therapy on the natural course of the INS. Gross changes

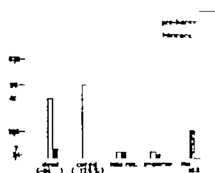


Fig. 4. Comparison of the results of th f obtained in the patients from pre-hormonal and hormonal (*black columns*) groups

are thereby considered, rather than ex ferences in percentages.

The following conclusions were reached fr this study. They confirm those of a previou one (13).

First of all it is obvious that steroid treatment prolonged to an important degree the duratio of the active disease in patients who rec vered ultimately. This is principally due to the oc currence of a higher number of relapses.

The second conclusion refers to the long-term effect of hormonal therapy on the natural cour of the INS. Without clinging too much to dif ferences in percentages, it can be said that the mortality rate decreased significantly that the number of healed patients increased and that multiple relapses are observed only in the hormonally treated group. As the number of cases with sequelae and late relapses apparently does not differ it could be postulated that as a consequence of hormonal (and antibiotic) treatment, those patients who would have died in the pre-hormonal era, now either heal or show multiple relapses. It cannot be said which effect is due t antibiot and hormone treatment respectively but it is tempting to identify the healed patients with those who would have died of infection in the pre-hormonal era. As far as the long-term effect of hormone therapy is concerned, the ultimate picture then depends on the evolution in the key-group with multiple relapses. When these patients will evoluate to renal insufficiency the main long-term effect of the hormone treatment will have consisted in an important postponement of the fatal issue in children who would have died i the pre-hormonal era. Will, on the contrary these patients

ultimately cure the long-term effect of hormone treatment will have been beneficial. Although the present investigations in these patients with multiple relapses, do not provide sufficient arguments in favor of such a fatal evolution, some histologic findings do not permit an optimistic approach. The multiple recurrences in patient II1 manifestly have resulted in a loss of glomerular material. It is not excluded, however that definitive healing still will occur with only partial loss of functional kidney tissue. A more alarming finding are the vascular lesions From this point of view patient II1 comes very close to patient IV1 another patient with a very long follow-up and who also had hypertension and showed vascular lesions on biopsy The role of long-standing or intermittent hyperlipidemia should thereby be considered. These two cases also clearly demonstrate the necessity for renal biopsy in children with multiple relapses, as in patient II1 only transient hypertension was observed and clearance values were normal in II1 as well as in IV1 after an evolution of more than 10 years. When we finally consider the pronounced glomerular lesions found in patient V2, a patient who undoubtedly would have belonged to the same group with multiple relapses, had he not died of a complication after 5 years of evolution, 3 pa- .. out of 10 who showed or still show multiple relapses, have pronounced histologic lesions of the kidney

As far as the long-term effect of steroids is concerned, one certainly has to await further follow-up studies, especially in those patients with *multiple relapses*. Meanwhile steroids remain indicated in a great number of patients with INS the selection of whom will not be discussed here. The fact that this treatment implies the risk of prolonging the activity of the disease in patients who in all probability will recover ultimately certainly arguments in favor of short trials with steroids.

Very scarce and contradictory literature data are available with regard to our first statement that hormone treatment prolongs the duration of the INS by enhancing the number of relapses. The same impression is gained from the results of a few similar studies (22). Other authors, on the contrary observed fewer relapses since intensive steroid treatment was used (6, 16). That fewer serious exacerbations are seen now is beyond doubt. Most authors who are in favor of short trials with steroids have in mind the potential side effects of prolonged therapy rather than the prolonged activity and the higher number of relapses brought about by such treatment.

The number of publications dealing with the statistical assessment of the differences in mortality and survival rate in pre-hormonal and hormonal era, is becoming unsurveyable. Differences in methods of investigation and definition of the different stages, do not allow a rigorous comparison of the figures. The length of the follow-up period determines to a large extent the durability of the results obtained. It is nevertheless possible to recognize the general trends that emerge from these studies. A first impression, gained from the study of patients with a follow-up of at least two years, is that the number of deaths decreased and that the number of survivings increased since hormone therapy was used. In other words that in these children life has been prolonged. Five years assessments still show the same trend but some authors draw the attention to the higher frequency of sequelae. Insufficient data are available concerning the further evolution after the first 5 years, but changes will undoubtedly occur Cornfeld, in a 10 years assessment, showed that after the first 5 years fewer children improve and that chronic changes of the kidney and deaths still occur (6). Although the experience gained from the present material is in accordance with these impressions, the small number of patients does not allow thourough conclusions. However this study draws the attention to some characteristics of the difference in survival and mortality rate From our data it appears in the first place that half of the patients who stay alive now show multiple relapses. At the same time it was demonstrated that important histologic lesions of the kidney can be found in a small percentage of those patients with the longest follow-ups. As a consequence careful follow-up studies in patients with multiple relapses will be of great importance in evaluating the long-term effect of hormone treatment. These studies were rarely done in the past and few histologic data are available. Worthen e.a. found normal kidney functions in 5 children with multiple relapses, and a normal renal histology on ordinary microscopy in 3 of them. Biopsies were performed 6 months, $5\,^{1}/_{12}$ years, $5\,^{3}/_{12}$ years and $1\,^{11}/_{12}$ years respectively after onset of the disease (28) Arneil mentions that in two children with 5

and 8 relapses respectively renal histology was normal (2).

Other aspects of the evolution of the hormonally treated INS, namely the influence of certain clinical, histologic and biochemical features at the onset of the disease and the interval between onset and treatment are also important but were not considered here.

SUMMARY

In 43 children with idiopathic nephrotic syndrome (INS) who were treated with ACTH or corticosteroids, the evolution was studied after 4 to 14 1/2 years. Frequent control investigations revealed that 29 patients were probably cured, 3 were dead, 2 showed a late recurrence and in 1 sequelae were found. In 7 patients, the NS was characterized by the occurrence of multiple relapses.

The comparison of these data with those of a prior identical follow-up study in 44 patients of the pre-hormonal era, permitted a gross evaluation of the influence of hormone therapy on the natural course of the INS

It was demonstrated that more hormonally treated patients cured, but that, due to the more frequent occurrence of relapses, definitive healing was significantly postponed. Less patients died, the number of late relapses and sequelae was not ery different in both groups. Multiple relapses were seen only in the hormonal group. The cured patients and those with multiple relapses thus probably represent the patients who would have died in the pre-hormonal era, but it is not known hich effect is due to antibiotics and hormones respectively

The long-term effect of hormone therapy will depend on the evolution in this group with multiple relapses. The histologic lesions in the kidney found in some of these patients suggest that probably in part of them, a f tal issue is to be ex pected. Further follow-up studies in the other patients with multiple relapses but with shorter follow-up periods, will reveal if identical lesions occur later in all these patients. If this is the case the long-term effect of hormone therapy in our patients consisted in an important postponement of the fatal issue

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ENDOGENOUS FORMATION OF CARBON MONOXIDE IN NEWBOR[illegible] TS

V On the Relation between the Carboxyhaemoglobin Concent ation an Haemoglobin Catabolism Calculated from Simultaneous Determinan of Carbon Monoxid Elimination and Total Haemoglobin

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In 1949 Sjöstrand reported the finding of increased concentrations of carboxyhaemoglobin (COHb) in the blood of adults with haemolytic diseases (25). Simultaneous determinations of the COHb level and the red cell survival in such patients have shown a good correlation between the COHb level and the degree of haemolysis (10 11). During the first week of life increased COHb concentrations have been found in infants with haemolytic disease of the newborn (14 15 21), and in infants with so-called physiological jaundice (1). At this age direct determination of the red cell survival is often impracticable. In the above-mentioned infants exchange transfusion is often necessary precluding erythrocyte survival studies. An evaluation of the COHb level as a quantitative measure of haemolysis would therefore be of interest during the newborn period.

Under certain prerequisite conditions the amount of carbon monoxide eliminated by the lungs (V_{CO}) can be assumed to give quantitative information about the haemoglobin catabolism, and with knowledge of the total amount of haemoglobin it allows calculation of the rate of haemoglobin catabolism. In this report the results of simultaneous determinations of COHb V_{CO} and total haemoglobin in full-term newborn infants are presented. An evaluation of the COHb level as an index of haemolysis has been made.

MATERIAL

Simultaneous determinations of the COHb concentration, CO elimination (V_{CO}) and total haemoglobin were performed on 32 full-term newborn infants. All inf nts ere in good condition and none displayed any clinical signs of respiratory disease. No infant was studied during the first day of life, if volatile anaesthetics other than nitrous oxide had been used at the delivery

The 32 infants comprised 12 full-term infants without blood group incompatibility and known haemolytic disease (Nos. 1–12, 15), 9 infants with jaundice and ABO incompatibility (Nos. 16–24), and 11 infants [illegible] Rh haemolytic disease (Nos. 30–40). In the latter group three mothers were smokers (Nos. 34–36) and two infants were studied after exchange transfusion (Nos. 37 and 38). The interval between birth or exchange transfusion and the investigation (33–76 hrs, mean 61 hrs) in these cases was so long that exogenous CO should not have influenced the COHb level to any appreciable degree, considering the efficient CO elimination found in full-term newborns (13)

All infants have been included in an earlier report dealing with the relationship between COHb and V_{CO} (13).

METHODS

Determination of the total amount of haemoglobin

General principle of he method. A modification of the open circuit CO-method (22) was used ith the infant attached to the system by means of nose mask [illegible] valves. The infant breathed a mixture of CO and air (CO concentration 0 0629 ± 0 0050 per cent) for period of 10 minutes. The amount of CO absorbed by the infant was calculated from the olume of air passing through the system during the CO breathing and the decrease of the CO concentration in the gas mixture. The COHb concentration in blood before and after CO breathing as measured. The total amount of haemoglobin was calculated from the increase of the COHb concentration and the amount of CO absorbed, using 1.34 ml CO per g haemoglobin as the CO combining capacity of haemoglobin

The open circuit breathing system. The same equipment was used for the determination of the CO elimination and the determination of total haemoglobin

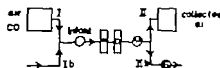


Fig 1 Breathing system used at the determination of the total amount of haemoglobin. *P* Filter with potassium hydroxide; *D* filter with soda lime; *M* membrane pump; *G* gas meter

breathing mask, the principle arrangement of the open circuit system (Fig. 1) and the bags for gas collection have been described in an earlier report (13).

Air with CO concentration in the above-mentioned range was sucked through the system (excluding infant) and collected in the way described below. On the average 96.4 per cent (S.D. = 3 per cent, $n = 9$) of the CO was recovered in the collected air. Thus small amounts of CO were "lost" to the system, despite the efforts to wash it out.

Procedure After blood had been drawn for determination of the initial COHb level, the room air sucked through the system (Connections I *b* and II *b*, Fig. 1) was replaced by the CO containing gas mixture (connection I *a*, Fig. 1). Collection of gas through connection II was started 10 seconds later. Connection I was closed after 10 minutes, but gas collection was continued for another minute and 10 seconds in order to wash out the system. Gas collection thus proceeded for period of 11 minutes. Blood for the second COHb determination was then drawn within 1 2 minutes.

The volume of the collected gas was measured by emptying the bag as completely as possible through gas meter type D 10 U The flow rate calculated from value agreed ell with the flow rate measured during earlier period by including the gas meter in the circuit connection II *b*, Fig. 1). The volume of CO containing sucked through the system was calculated from the volume of the collected gas by multiplication with 10/11

Determination of the CO concentration in air

CO concentrations in air in the above-mentioned range were determined according to Linderholm & Sjöstrand (16). Each sample was analysed in duplicate. The random error of single determination was calculated from the duplicate determinations to be ± 0.0065 per cent CO or ± 0.9 per cent of the mean value

Determination of the COHb concentration in blood

The COHb concentration was determined according to Linderholm *et al* (17). The random error of single determination has earlier been calculated from duplicate determinations to be ± 0.05 per cent COHb

Calculations

In the calculation of the amount of CO absorbed by the infant, the CO concentration in the collected air as corrected for CO "lost" to the system by multiplication with the factor 1/0.964 (= 1.037). The oxygen consumed by the infant was not replaced by added carbon dioxide, since the expired carbon dioxide was removed by the filter in the system. In the calculation of the volume of air sucked into the breathing mask correction was therefore made for oxygen consumption, estimated to be 6 ml per kg and min (20).

N correction was made for CO bound to myoglobin and other non-haeme compounds (see below).

The total amount of haemoglobin was then calculated from the formula

$$\text{Total haemoglobin} = \frac{100\left[\left(\frac{10}{11}V + \quad\right) - Vb\,1.037\right]}{1.34(\text{COHbII} - \text{COHbI})}$$

where V = measured volume of collected air in ml STPD, = correction for oxygen consumption, = initial CO concentration in the air in per cent, b = measured CO concentration in the collected air COHbI = initial COHb level in per cent, and COHbII = final COHb level.

Random error of the method

The total amount of haemoglobin was determined twice with 1 2 day intervals in 10 infants aged 2 10 weeks. All infants were in good general condition and none had signs of infection or haematological disease. No systematic difference was found between the first and second determinations. The random error of the method calculated from the duplicate determinations was found to be ± 5.5 g, or ± 14 per cent of the mean.

Determination of the pulmonary CO elimination

The method for determination of the Vco has been described in an earlier report (13). Duplicate determinations were done with a random error of single determination f $\pm$ .6 µl CO per 10 min.

Ordinary statistical methods were used to calculate mean values, standard deviations and linear regression coefficients (28).

The relationship between the COHb level and the rate of haemolysis was analysed by computer (IBM 360). Different regression curves were tested according to the method of least squares (6). The goodness of fit as estimated from the ratio variation ttributable to regression/total variation

RESULTS

Table 1 shows the increment of COHb and the amount of CO absorbed during CO breathing, and calculated total haemoglobin in each infant, together with the initial COHb level, the pulmonary CO elimination, the venous haemoglobin concentration and the weight of the infant.

In two infants (Nos. 15 and .4) the estimated CO absorption per kg body weight (1 049 and 1 766 ml respectively) markedly surpassed the amount absorbed by the other infants (mean 0 471 ml, S.D. 0 1 6 ml). In both these cases very low CO concentrations were found in the collected air

AB Nordgas, Stockholm.

Table 1 COHb increment and amot f absorbed CO during CO breathing ith calc tal haemoglobin in each infant together with u l COHb level, CO elimination per unit time moglobin concentration and body weight

Case no.	Weight, kg	Venous haemoglobin, g per 100 ml	Init l COHb,	COHb increase, %	Absorbed CO ml per kg	Total haemog g	ir	at on, 0 min
2	3.34	20.8	0 8	1.90	0.675	89		
3	3.65	21.8	0 66	2.95	0.652	60	9	
4	2.80	22.3	0 64	3.46	0.776	47	6	
5	3.44	18 0	0 85	2.43	0.223	24		
6	3.17	20.2	0 78	2.27	0.475	49	6 5	
7	3.90	21.8	0 81	1.26	0.277	64	4 3	
8	3.48	20 6	0 99	1 72	0.410	62	10 3	
9	2.72	22.0	1 02	1 77	0.363	42	11 2	
10	2 96	22.3	0.89	.21	0.511	51	7 3	
11	3.86	17 9	1 53	3.82	0.484	37	14 8	
12	3.19	20.3	1 04	1.68	0.571	81	12 4	
15	4 00	18.6	1 38	1.8	(1.049)[a]	(158)[a]	19.8	
16	2.9	1.0	0.91	1 67	0.582	76	6.4	
17	3.80	22.4	1 07	3 01	0.648	63	12.2	
18	3 78	18.9	1.09	2.70	0 407	42	8 4	
19	2.98	17.5	0 97	1 73	0 470	60	8.3	
20	4 11	18.4	1.39	2.17	0.366	52	13 7	
21	2.81	19.0	0 86	2.43	0.526	45	0	
22	3.08	19.3	1.03	2.07	0 604	67	6.5	
23	3 70	16.9	2.16	.96	0 407	38	.2.	
24	3.48	17 7	1.69	3 70	(1 766)[a]	(1 4)[a]	13 7	
30	2.51	11 4	2.20	3.80	0.414	70	10 0	
31	3.68	70 0	1 73	2.31	0.473	56	21 9	
32	3 77	15.9	1.20	3.05	0.369	33	9 0	
33	3.40	15.5	1 12	4.33	0.574	34	11 6	
34	2.34	14.4	1.51	4 12	0 422	18	14 0	
35	3.00	14 1	1 40	3 06	0 363	27	1 0	
36	3.36	14.3	0.82	5.93	0.457	19	9 6	
37	3.22	16 0	1.83	3.81	0.516	33	10 2	
38	2.98	14.7	1 1	2.68	0 378	31	8.7	
39	3.34	20.0	1 15	2.62	0 3 4	31	10 5	
40	3 48	19 6	0.87	2.26	0 393	45	10 9	

[a] Values excluded because of probable leakage in the breathing system.

suggesting leakage of room air into the system. The calculated total haemoglobin in cases 15 and .4, 158 and 124 g respectively are unreasonably high. The two values have therefore not been included in the following calculations.

Table . gives the amount of haemoglobin per kg and the blood volume per kg calculated from the haemoglobin mass and the enous haemoglobin concentration using 0 91 for the relation between body haematocrit and venous haematocrit (3). The two infants with Rh haemolytic disease investigated after e cha ge transfusion have not been included. A significant correlation ($r = 0\ 66$) was found between the enous haemoglobin concentration (x) and the amount of haemoglobin per kg (y) The calculated regression equation is $\hat{y} = 1_8\,x - 9.6$

A significant correlation was found between COHb and V_{CO} per g haemoglobin and unit time ($-0\ 69$) (Fig 2, Table 3). As comparison the results f three similar investigations on adults are given (8, 10 11). In one of these the CO production nd total haemoglobin were measured directly (8), in the others the CO production w calculated from the results of red cell surv l studies by the present author (10, 11) The results of the four investigations suggest curvilinear relation between the two variables, and analysed together by a computer they were found to fit best the following two equations, $y = 3.3$ – .75 and $y = 0.14 - 2.13\,x^{1}$ ($x = V_{CO}$ in µl per g haemoglobin and 10 min, $y =$ COHb in per cent, $e =$ base of natural logarithms). It can be estimated that the COHb value corresponding to.

Table 2. *Mean values for haemoglobin and blood volume per kg body weight in full-term newborns*
Data from the present study compared with some earlier investigations

Author	Method	Age (hours or days)	Haemoglobin (g per kg)	Blood volume (ml per kg)
Mollison *et al.* (1950)	^{32}P + T−1824	0– 4 h		84.7
Fasham *et al.* (1950)	^{32}P	0–24 h		94.9
Sisson *et al.* (1959)	T−1824	0–24 h	17.0	98.2
		4 d	15.6	97.8
Usher *et al.* (1963)	^{131}I	4 h		75.3[a]
		1 5 d		82.3[a]
		2–6 d		92.6[b]
Bratteby (1968)	^{51}Cr	0–7 d		87.9[b]
Present study	CO			
Rh haemolytic disease		0–4 d	9 6	65.3[a]
ABO incompatibility		0–6 d	16 8	96.0[b]
No incompatibility		3–6 d	16 7	88.2[b]

[a] Early clamping of the cord.
[b] Late clamping of the cord.

$V_{CO} = O$ should be of the same magnitude as that given by the second equation (see below). Therefore an equation of that type was chosen as the best expression for the relationship between the two variables.

The regression equation calculated from the results of the present investigation on newborns is

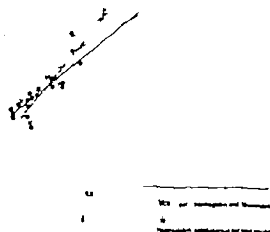


Fig The relationship between the COHb level and the rate of haemolysis found in the present investigation on newborns (large filled circles, coarse line) and in three earlier studies on adults (Engstedt (1957)—crosses, coarse broken line; Coburn *et al.* (1965)—small filled circles, fine line; Coburn *et al.* (1966)—small open circles, fine broken line). Regression curve, calculated from the results of all four investigations—curved broken line

described by $\bar{y} = 0.31 + 1.71\,x^{1}$ Fifty per cent of the variation in the material could be attributed to the regression. The standard error of an estimate was calculated to be 0.30 According to the equation a red cell survival of 1_0 days (equivalent to the production of 0 078 µl CO per g haemoglobin and 10 min) on the average corresponds to a COHb level of 0 78 per cent, 60 days red cell survival to a COHb level of 0.98 per cent and 30 days survival to 1_6 per cent.

DISCUSSION

All methods for determination of the total amount of haemoglobin require the introduction of a tracer substance into the blood. For several reasons a CO-method was chosen for the present study A reliable method for analysis of the CO content in air and blood was available The procedure allowed the use of the same breathing system as in the preceding determination of the CO elimination and only one additional venipuncture was necessary Since some infants had to be investigated immediately prior to an exchange transfusion, it was advantageous that the determination of total haemoglobin could be accomplished rapidly

The calculation of the total amount of haemoglobin required determination of the increase of the COHb concentration in blood during CO-breathing and of the amount of CO absorbed during the exposure.

In the present investigation the mean increase

Table 3 Relationship between COHb and rate of haemolysis found in the present invest   newborns compared with earlier investigations on adults

Methods employed and results

	Methods						
	COHb (mean value ± s.d. in healthy adults)	Rate of haemolysis	Number of cases	Regression equation*			ation ent
Engstedt (1957)	With rebreathing system. Hopcalite method (0.54 ± 0.13)	Red cell survival	18	$y = 2.44x + 0.51$	0.4	0	
Coburn *et al.* (1965)	Direct in blood. Infrared method (0.88 ± 0.11)	CO production	13	$y = 1.09x + 1.09$	0.45		
Coburn *et al.* (1966)	Direct in blood. Infrared method (0.88 ± 0.11)	Red cell survival	8	$y = 1.29x + 1.18$	0.46	0	
Present study	Direct in blood. Hopcalite method (0.72 ± 0.09)	CO elimination	30	$y = 1.62x - 0.72$	0.30	0.65	

V_{CO} µl per g haemoglobin and 10 min, y = COHb per cent.

of the saturation of haemoglobin with CO was 2.74 per cent. From the random error of a single COHb analysis it can be calculated that this increase was determined with an error of ±0.07 per cent COHb. Blood for determination of the final COHb level was drawn 2–3 minutes after the infant ceased breathing CO. This interval should allow for a fairly even distribution of the CO in the blood. During the first 70 seconds after CO breathing the infant was still attached to the breathing system and air was collected. The CO in the respiratory tract can therefore be assumed to have been either absorbed or expired into the system. The amount of CO lost to the ambient air before blood sampling was estimated to be negligible.

Although the error in the determination of the COHb increment was small the random error of a single determination of the total haemoglobin was considerable, amounting to ±14 per cent. The estimation of the amount of CO absorbed by the infant must therefore be crucial for the accuracy of the method. The CO absorption was calculated as the difference between the CO content in the air sucked into the breathing mask and in the collected air. The measurements included in this calculation were performed with high precision, but the combined effect of their errors obviously was considerable. The corrections for oxygen consumption and for CO lost to the system, aiming to eliminate systematic errors, introduced additional random errors.

In all CO-methods leakage of the breathing system invalidates the determination of total haemoglobin. Because of the position of the pump in the system used in the present investigation the probable effect of a leak would be an entrance of room air leading to an overestimation of the CO absorption and calculated total haemoglobin. It was found that the CO absorption per kg varied comparatively little between the infants, with two exceptions (Table 1). The very high values found in infants nos. 15 and 4 probably indicated leakage.

No correction was made for CO bound to non-haeme compounds. It has been estimated that in adults about five per cent of the CO is bound to compounds other than haemoglobin, mainly myoglobin (24). I newborns the muscular mass is relatively small and the error caused by CO bound to non-haeme compounds can be assumed to be of less importance than in adults.

Despite possible systematic errors, the mean values for the amount of haemoglobin and/or calculated blood olume per kg found in the present material, except in infants with Rh haemol

disease, agreed fairly well with those reported earlier (5 12, 19 23 30) (Table 2). Furthermore, the equation of the linear regression between amount of haemoglobin per kg and venous haemoglobin concentration agreed with reported equations of the linear regression between red cell volume per kg and venous haematocrit (4). In Rh haemolytic disease considerably lower values were found both for the amount of haemoglobin and the blood volume per kg. Even accounting for the early clamping of the cord, these results do not indicate that a compensatory increase of the blood volume had occurred. This is in agreement with the finding reported by Mollison *et al* that compensatory increase of the blood volume in heamolytic anaemia is not seen until the venous haematocrit has fallen below 25–30 per cent (19) Anaemia of that degree was not seen in the present cases of Rh haemolytic disease.

Quantitative information about the haemoglobin catabolism can be obtained from the pulmonary CO elimination under certain prerequisite conditions. Endogenously produced CO must be assumed to be derived mainly from degraded haemoglobin and to be eliminated quantitatively in the expired air Furthermore a steady state is assumed with constant CO production, and constant CO concentration in the inspired air

Sjöstrand has shown that the pulmonary CO mination in healthy adults is accounted for by normal decay of circulating haemoglobin (26) adults with haemolytic diseases Coburn *et al* nd a good correlation between the rate of CO oduction and the rate of haemoglobin catabolism calculated from Cr^{5}-labelled erythrocyte decay curves (10). In certain pathological conditions other sources for the CO production must be considered, but there is no information about this possibility in newborns.

Oxidation of CO to CO_2 has been shown to occur in animals (7 18) and in man (18 29). The rate of oxidation is low at COHb levels in the actual range (Table 1) Furthermore, the oxidation is thought to occur in skeletal muscle, which should reduce its importance in newborns still more.

The influence of variations in inspired CO probably was small since the room air CO concentrations were comparatively low in this study (13).

Considerable variations of the COHb level can be found in individual infants during the newborn period (1 14 15). In the case of increasing CO production the COHb level and the CO elimination have been found to rise rapidly (9 27), and can be assumed to reflect the increasing CO production closely After a decrease of the endogenous CO production, on the other hand, there is a time lag of several hours before the new steady state is reached because of the slow elimination of CO

The above-mentioned prerequisites probably were fulfilled approximately in the infants included in the present investigation and the CO elimination per g haemoglobin and unit time has therefore been assumed to give an estimate of the rate of haemolysis.

A significant correlation was found between COHb and the rate of haemolysis (Fig. 2, Table 3). Despite methodological differences the results of the present investigation agreed fairly well with the results of three similar studies on adults. A comparison of the four regression lines demonstrated that the regression coefficient was smaller when the material included cases with more severe haemolysis. This finding is compatible with a curved regression of the rate of haemolysis on the COHb level. Other facts also speak in favour of a curved regression. The intercept of the regression curve on the y axis can be assumed to correspond to the COHb level caused by the small CO concentrations in the ambient air According to Haldane's equation the CO concentrations found in room air in the present study would give an average COHb level of 0 15 per cent. The intercept of the regression line was far in excess of this value. Bjure *et al* have found a positive correlation between the pulmonary diffusing capacity for carbon monoxide and the pulmonary blood volume (2). Thus the increased blood volume found in severe haemolytic anaemia might allow CO elimination to be achieved at a COHb level not increased in proportion to the rate of haemolysis.

The results of the four investigations were therefore analysed together by a computer and a regression curve, assumed to describe the relationship between COHb and rate of haemolysis was selected with the guidance of the consistency of the results and the magnitude of the intercept. It is not claimed that this regression curve describes the true relationship between the two variables.

however, it probably is a better approximation of this relationship than the linear regression. If we want to use the regression equation to predict the rate of haemolysis from the COHb level, the standard error of the estimate must be considered. Large individual differences were found in the relation between the two variables. This variation was partly caused by the random errors of the measurements employed. The error in the estimation of haemolysis was considerable, exceeding 20 per cent. The COHb concentration, on the other hand, was determined with fairly high precision. Thus, there are reasons to believe that the COHb level is a better index of haemolysis than that appears from the relationship between the two variables found in this investigation.

SUMMARY

A modification of the open circuit CO-method was developed for determination of the total amount of haemoglobin in newborn infants. Simultaneous determinations of the COHb level, the pulmonary CO elimination and the total haemoglobin were performed on 32 full-term newborn infants. The rate of haemoglobin catabolism was calculated from the CO elimination and the total haemoglobin on the assumption that CO is formed mainly from degraded haemoglobin and is eliminated quantitatively in the expired air.

An evaluation of the COHb level as a quantitative measure of haemolysis was made.

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LATE INFANTILE PROGRESSIVE ENCEPHALOPATHY WITH DISTU[illegible] UNSATURATED FAT METABOLISM

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This report concerns preliminary notes on a patient who died when six years old and where combined clinical, histological, histochemical and biochemical examinations appeared to reveal a previously unknown disorder with late infantile onset, viz. symptoms and signs of a severe progressive encephalopathy pronounced changes in both the grey and white matter and a remarkable disturbance of the brain lipids, predominantly implying a disturbed metabolism of linolenic acid in the brain.

CASE REPORT

H. L. Record no. UAS 1941/63 This girl, born March 15 1961 was the only child of healthy non-related parents. She had a healthy half-sister born 1949 Pregnancy and delivery were uncomplicated. She was born at the expected time with a birth weight of 3208 g. The neonatal period was uneventful. She started to grasp when 4 months old, sat at 7 months, crept at 9 months, and walked with slight support at 1[illegible] months, but never learnt to take more than a few steps unaided. At an age of about 1 year she could speak single words and even single simple phrases. After that time her speech successively deteriorated to unintelligible syllables and at the same time she began to become dull and inactive and seemed first to arrest and then regress in her motor development. When $1\,^1/_2$ years old she was seen by the local pediatrician, who found an unsteady and broad gait and signs of truncal ataxia. Developmental tests according to Gesell revealed a developmental age of 40–44 weeks in her upper and 5[illegible] weeks in her lower extremities. The adaptive level wa[illegible] at 40–48 weeks of age, speech at 36 weeks and social behaviour [illegible]t 36–40 weeks. In summary she had a developmen[illegible] [illegible] of 7–9 months. Special examinations o[illegible] [illegible]e ears were normal, as were the EEG and th[illegible]

When $1\,^3/$[illegible] years old nodding spell[illegible] [illegible]n appeared 20–30 times a d[illegible]y Her me[illegible]tal r[illegible] sion increased and she had no longer any co[illegible] [illegible] with her parents. She was first se[illegible] in the pediatric department of Uppsala [illegible]t the age of [illegible] years. At that time she was severely [illegible]tarded had no speech or grasping ability coul[illegible] [illegible]it but not stand by herself and had a pronoun[illegible]d t[illegible]ncal ataxia. Her reflexes were normal and she [illegible] no optic atrophy However at the same time [illegible] seemed to have an impaired vision and did not react in a normal way to optic stimuli. Her EEG which had been normal 5 months earlier now showed severe and diffusely spread abnormalities with an irregular delta activity and bilateral synchronous sharp waves of a low amplitude.

She was now transferred to a home for the severely mentally retarded and her deterioration further progressed. When [illegible]$/_2$ years old she did not even make any sound and could no longer sit. Soon after that she lost the ability even to turn around from prone to supine and vice versa. Her eye movements became irregular and erratic and her fits increased in frequency and intensity When [illegible] [illegible] years old she was again brought to the pediatric department of Uppsala for further examination. She was now found to be soporous and in a neonatal motoric state with a flexion pattern, no head control and a generalized floppiness. Ankle clonus was consistent bilaterally and her pla[illegible]tar response was extensor Her eye movements were nystagmoid, she reacted to light but the pupillary response was slow in both eyes. The fundi were still normal. Yet more progressive ab-

acid patterns for cerebral cort. and white matter and the pronounced diminution of fatty acids of the linolenic acid series (ω3-series). In normal brains the ratio saturated monoenoic acids is much larger in grey than in white matter but in the present case the same ratio was found for the two sources and it was very similar to the ratio in normal white matter There were very low concentrations for 22 6ω3 and 22.4ω6.

The cholesterol esters were slightly increased and the fatty acid pattern differed from the normal by a very large concentration of oleic acid (50%) and very low concentration of polyenoic acids. The pool of free fatty acids was also increased palmitic acid and oleic acid comprised about 30% and stearic acid and arachidonic acid 10% each.

In the present case the fatty acid patterns of the phosphoglycerides were seriously changed. A pronounced diminution of polyenoic acids derived from linolenic acid was the most remarkable finding. It has in general been assumed (4) that the two enzyme systems, the chain lengthening and the desaturating, are common for the fatty acids of the linoleic and linolenic acid series. The concentration of arachidonic acid (20 4ω6) was normal or slightly increased which would indicate functioning enzyme systems. Neverthe less, the present figures indicate that there might different enzyme systems for the two fatty acid ies and that those for linolenic acid were sed, but it is impossible to decide whether the changes were primary or secondary When linolenic acid is not metabolized normally it will be susceptible to oxidation, and it is very probable that the granulate fluorescentic PAS-positive material seen in the sections are oxidized fatty acids of the linolenate series.

DISCUSSION

The rapidly developing clinical picture with early mental retardation, loss of speech, minor motor seizures, regression of motor development and ataxia together indicate a severe and widespread involvement of the brain. The cortical biopsy suggested some form of neurolipidosis well in agreement with the clinical symptoms and signs. However at autopsy known forms of neurolipidoses (7), leucodystrophies (5) poliodystrophies (7) and axonal dystrophy (1) could be excluded for combined histological, histochemical and biochemical reasons. Dominating histological features were total derangement of cortical cytoarchitecture, severe degeneration of white matter and deposits in both grey and white matter of a granular material with histochemical properties characterizing free fatty acids and unsaturated polymerized and oxidized fatty acids. This could well be in agreement with the seriously changed pattern of fatty acids demonstrated at the biochemical investigation and mainly indicating a disturbance of linolenic acid metabolism.

Until recently no genetic errors in fatty acid metabolism have been described. In 1967 Sidbury *et al* (6) reported two families with infants who had had an odor of sweaty feet, lethargy convulsions, acidosis, a defective butyric and hexanoic acid metabolism, and who died during the first months of life. Our case differs from this disease in all essential respects. It might, however be another example of a disorder primarily affecting the metabolism of fatty acids. If so, it represents a more chronic form, dominated by involvement of the central nervous system and with obvious similarities to the neurolipidoses.

SUMMARY

A case report is presented of a 6-year-old girl with a severe progressive encephalopathy of late infantile onset and with changes indicating marked disturbances in the metabolism of linolenic acid.

ACKNOWLEDGEMENTS

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FIBRINOLYTIC TISSUE ACTIVITY IN THE UROGENITAL TRACT AND THE ADRENAL GLAND OF HUMAN EMBRYOS AND NEW BORN

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Investigations of the concentration of plasminogen activator in embryonic tissues are few. Permin (.8) and Albrechtsen (1) compared the activator activity in various tissues from calf embryos and the adult ox. Lung tissue from infants dying perinatally some of whom with hyaline membrane disease was studied by Lieberman (24), Lieberman & Kellogg (25), Kloos & Libal (18) and Ambrus *et al* (3) and an investigation of the development of pulmonary fibrinolytic acti ity during foetal life and postnatal period in humans and guinea pigs was published by Ambrus (4). A comparative study of the fibrinolytic activity in various organs from and adult human cadavers was made by *et al* (33) and an in estigation of human embryonic tissues in the first trimester of pregnancy was reported by L szynski *et al* (35).

The present p per reports an investigation of the content of pl sminogen activator in the various tissues of the urogenital tract and in the adrenal gland from human fetuses and premature or still-born infants. A similar study of the urinary tract in humans of different ages, ranging from 1 to 94 years, has been published previously (21).

MATERIAL AND METHODS

The tissue samples were obtained from 15 fetuses and 7 premature or still-born infants. The ages of the former ranged from 15 to .4 eeks of pregnancy and of the latter from 26 to 38 weeks. The fetuses were all obtained at hysterotomy in cases of legal abortion and they were immediately cooled at 0°C to avoid autodigestive activity. In the 7 cases of premature and still-born infants the tissue samples were obtained at autopsy.

As far as possible samples were taken from all parts of the uro-genital tract: renal cortex and medulla, pelvis, ureter, esical mucosa and musculature, prostate, testicular tunica albuginea and parenchyme, epididymis, uterus, ovary and from the adrenal gland. In several cases the small size of the organs made such a detailed dissection impossible if at the same time samples of quantity sufficient for analysis were to be obtained, for which reason samples were taken from kidney parenchyma as a hole, from pelvis-ureter, bladder and testis. The tissue sample ere investigated at once or after storage at −20°C for few days.

The concentration of plasminogen activator was estimated by the quantitative method of Astrup & Albrechtsen (5). The sample was homogenized and the activator extracted with 2 *M* potassium thiocyanate. After precipitation at acid reaction (pH 1) and redissolution the fibrinolytic activities of serial dilutions were estimated on unheated bovine fibrin by the plate method (7) and a dilution curve was recorded. By interpolation upon standard curv obtained simultaneously the concentration of activator was expressed in units of the pig heart standard preparation per gram fresh tissue.

The possible interference of unspecific proteolytic activity which might occur especially in the autopsy material, was tested on heated fibrin plates (23). Since no activity was found the activities estimated on normal fibrin exclusively represent activator activity.

Because of the very small tissue samples, sometimes only 5 mg, the original amount of 15 ml KSCN used for extraction was often reduced in order to secure a concentration of the extract, sufficient to obtain reliable estimation on the fibrin plates.

RESULTS

Based upon about 2000 single estimations the concentrations of plasminogen activator in respectively the urinary tract and the genital tract and

This investigation was supported by grants from the Danish Foundation for the Advancement of Medical Science and from the Association for Aid of Crippled Children, New York.

Table 1 Concentration of plasminogen activator in the urinary tract of 15 human fetuses and 7 premature or still-born infants

The figures represent U/g fresh tissue

No.	Sex	Age (weeks)	Kidney Cortex	Kidney Medulla	Cortex and medulla together	Pelvis	Ureter	Pelvis and ureter together	Bladder Mucosa	Bladder Musculature	Bladder as a whole
1	M	15			556	7	10				380
2	F	15			204			470			457
3	M	16			216	61	50				145
4	M	16			1086			228			540
5	F	16			245			161			560
6	M	17	137	887		101	142				405
7	F	17			390			142			389
8	F	17			532			71			468
9	F	17			210			180			60
10	M	18	252	800				112			575
11	M	19			425	127	25				303
12	M	20			475	121	186				370
13	F	20			765			[illegible]			[illegible]
14	F	20	248	378				140			341
15	M	24	314	451				501			720
Average			239	629	646	83	83	270			419
16	M	26	590	545				557	357	160	
17	M	29	189	298				332	185	[illegible]	
18	F	32	375	661				672	217	139	
19	M	35	101	289		368	364		335	290	
20	M	36	200	200				1160	360	255	
21	M	38	228	186		1200	1278		800	480	
22	M	38	82	258		404	194		203	64	
Average			232	348		658	612	680	354	207	

adrenals are seen in Tables 1 and 2. The Nos. 1–15 include the hysterotomy material and the Nos. 16–22 the autopsy material.

Urinary tract

All parts showed moderate or high fibrinolytic activity.

In kidney parenchyma moderate activity was found in the cortex and equal in both materials. A large activity appeared in the medulla and especially in the second trimester of pregnancy which may be the cause of the high activity of kidney parenchyme as whole in the hysterotomy material the majority of the tissue sample containing of medulla.

Pelvis and ureter were almost equally active but apparently increasing values were found with increasing age of gestation. The concentration of activator in the bladder as a whole in the second trimester was nearly the same as in the mucosa in the last trimester which, however was larger than in the corresponding bladder musculature.

Genital tract and adrenal

Prostatic tissue showed moderate to large activity increasing with the age of gestation. Moderate activity was found in testis as a whole in the second trimester as well as in testicular parenchyme up to term, whereas higher activities appeared in epididymis and tunica albuginea.

Uterus and ovary showed moderate or large content of activator in the ovary ranging from 91 to 5490 U/g, however.

Small concentrations without much spread were found in the adrenals in all cases.

DISCUSSION

As in all previously published investigations of fibrinolytic activator activity in tissues and organs the appearance of large individual spread of the values in all tissue groups limits the usefulness of average values in the present study. However the fairly constant relation which was found between the activities of the different tissue

Table 2. *Concentration of plasminogen activator in 7 premature or still-born infants*

The figures represent U/g fresh tissue

			Testis	
No	Age (eeks)	Prostate	Epididymis	Tunica flug.
1	15	10		
2	15			
3	16	51		
4	16	15		
5	16			
6	17	128		
7	17			
8	17			
9	17			
10	18	131		
11	19	,53		
12	20	570		
13	20			
14	20			
15	24	288		
Average		198		
16	26	455	335	3 6
17	29	342	455	336
18	32			
19	35	350	449	479
20	36	707	211	166
21	38	804	236	131
22	38	164	142	2,6
A erage		470	305	277

may justify their mutual comparison as well as a arison with previous investigations of em lc and adult tissues in humans and animals.

Compared to urinary tissues from humans, aged 1–94 years (21) he fetal kidney had a higher activity both in ortex and medulla, but in both materials medulla was most active. The activity of pelvis and ureter appeared to increase during fetal life, reaching that of the adult organism, whereas the fetal bladder was a little less active, but with the same relation between mucosa and musculature, the former being more active.

Sandberg *et al* (33) using a semiquantitative estimation method of f brinolytic activator content, found no difference between renal tissues from infant and adult humans, but early fetal tissues were not investigated, further more were cortex and medulla not studied seperately

Among other tissues from humans, aged 0–82 years, Albrechtsen (2) investigated the activator content in male and female genital organs and Rasmussen *et al* (31) studied adult myometrium.

Table 1 *Clinical data on the cases*

	Normal	Toxemia	Rh-immunization	Prolonged pregnancy
No. studied before labor	12	8	9	8
Total no. studied	12	11	9	10
Primiparous	3	7	—	7
Multiparous	9	4	9	3
Gestation, days				
Mean	272	276	65	291
Range	264–290	255–299	261 269	285–296
Birth weight, g				
Mean	3,363	2,845	3,230	3 632
Range	2340–4380	1890–4580	2570–3590	3300–4070
Complications of delivery	1 Cesarean section 1 Vacuum extraction 1 Twins	1 Vacuum extraction 1 T plets	—	1 Cesarean section 2 Vacuum extraction

IV *Prolonged pregnancy* Duration of pregnancy for 10 or more days after the expected date and/or positive radiological evidence (placental calcification and/or advanced fetal skeletal age) were considered as criteria for this group of ten cases. Cesarean section was performed in one case because of cervical dystocia associated with fetal acidosis (pH 7.26, standard bicarbonate 15.8 mEq/l), but no other acid-base disturbances were observed. The Apgar score was 8 or more in all cases at five minutes. One infant was noted to have cleft lip and esophageal atresia, and as operated at the age of 12 hours. For additional information see Table 1

Methods of sampling

Fetal blood, obtained by pricking the hyperemic scalp, as sucked into long glass capillary tube containing heparinized thread. Aliquots of the contents of the capillary were immediately precipitated in triplicate with sodium hydroxide and zinc sulphate. Maternal capillary blood was simultaneously collected from fingertip and treated similarly

After the basal values prior to the onset of labor had been obtained, further samples were taken at intervals as labor progressed. In 32 cases the umbilical cord as double clamped immediately after delivery before the first breath, and samples of arterial and venous cord blood were separately precipitated.

Chemical methods

The concentration of glucose in the supernatant fraction f the precipitated samples as determined with specific enzymatic method (11), using glucose oxidase eagent obtained from AB, Kabi, Stockholm. The parameters of acid-base balance in blood ere determined with the method of Astrup *et al.* (1), using an apparatus supplied by Radiometer Copenhagen.

RESULTS

Assessment of the value of capillary blood glucose determinations

W have tried to assess the value of information obtained from the scalp capillary samples by comparison of the glucose levels with those measured in the umbilical vessels. Of course, such a com-

Table 2. *Comparison of the glucose concentration in fetal scalp capillary blood, obtained immediately before birth, to the values in the umbilical artery (UA) and vein (UV)*

Fetal capillary blood mg per 100 ml	Min before birth	UA (mg per 100 ml)	UV (mg per 100 ml)	Difference (capillary/UA) mg per 100 ml	of UA
83	1	79	89	4	5
74	1	70	84	+4	6
64	3	63	71	1	2
80	4	74	84	+6	8
69	5	62	92	+7	11
67	6	62	77	+5	8
75	6	84	92	−9	11
61	7	70	86	9	13
75	10	78	91	3	4
Mean 72		71	85	4.3*	7.6

Mean absolute capillary/UA difference (mg per 100 ml).

Table 2. *Concentration of plasminogen activator in the genital tract and andrenals of 15 human fetuses a— 7 premature or still-born infants*

The figures represent U/g fresh tissue

N	Age (weeks)	Prostate	Testis: Epididymis	Testis: Tunica albug.	Testis: Parenchyma	Testis: Testis as whole	Uterus	Ovary	Adre
1	15	10				84			78
2	15						1050	168	97
3	16	51				89			32
4	16	152				422			106
5	16						12..	5490	115
6	17	128				203			0
7	17						190	65	40
8	17						364	108	91
9	17						560	210	53
10	18	131				151			54
11	19	253				203			143
1	0	570				72			94
13	20						659	248	120
14	20						362	91	54
15	24	288				280			48
Average		198				188	472		85
16	26	455	335	326	280				227
17	29	342	455	336	117				55
18	32						774	330	100
19	35	350	449	479	206				63
20	36	707	211	166	61				30
21	38	804	236	131	37				17
.2	38	164	14	226	103				+
A erage		470	305	277	134				70

may justify their mutual comparison as well as a comparison with previous investigations of em- ... adult tissues in humans and animals. ... to urinary tissues from humans, aged ... (.1) he fetal kidney had a higher ac ... ivity in ortex and medulla, but in both ... enals medulla was most active. The activity of pelvis and ureter appeared to increase during fetal life, reaching that of the adult organism, whereas the fetal bladder was a little less active, but with the same relation between mucosa and muscu lature the former being more active.

Sandberg *et al.* (33), using a semiquantitative estimation method of fibrinolytic activator content, found no difference between renal tissues from infant and adult humans, but early fetal tissues were not investigated, further more were cortex and medulla not studied seperately

Among other tissues from humans, aged 0–82 years, Albrechtsen (?) investigated the activator content in male and female genital organs and Rasmussen *et al* (31) studied adult myometrium

Compared with these investigations, the sm estimation method being used in the present pape the activity of the prostate appeared to increa during fetal life reaching the large activity of th adult gland. Large activities were found in bo the fetal and the adult uterus and ovary where the fetal testis was more active than the adult on Investigations of the adult epididymis and tuni albuginea, however are not available.

In contrast to the large average content 410 U/g in adult adrenals (2) there was only litt activity in fetal adrenal tissue and this differen was also found by Sandberg *et al* (33).

During the first trimester of pregnancy the a tivity of human embryonic tissues, without org differentiation was even larger than the high a tivity in non-pregnant uterine tissues (35).

Fibrinolytic activator investigations of calf em bryos and adult ox (1 28) showed that the activi disappeared from kidney and lung shortly aft birth but remained unaltered in testis and adrena

Because of the possible role of the fibrinolyt

seem in the pathogenesis of hyaline membrane ... especially lung tissue has been studied in embryos and premature and mature infants (3 ... 4, 25) and in a detailed study of human and guinea pig embryos (4) an increase in pulmonary activity appeared up to term, followed by a decrease to the adult range in the postnatal period. The increase before term was considered a physiologic protective mechanism against pulmonary fibrin deposition.

In the fetal human genito-urinary tract the activator content in kidney parenchyme and testis was higher than in adults, whereas in the majority of the other tissues similar high activity appeared as in the adult organism in contrast to the little active fetal adrenal. The cause and the possible physiological importance of the difference in fetal and adult fibrinolytic activity which has been found in some tissues cannot be explained. It might be related to differences in vascularization, since tissue activator is highly localized to the endothelial cells of blood vessels (19 34), but other cell types can exhibit fibrinolytic activity (6). Plasminogen activator has been found in supernatants of kidney tissue cultures (8 27) and the activity in renal cortex has been localized especially to cells of the juxtaglomerular apparatus (29).

The high activity in the fetal urinary tract might be related to the occurrence of the plasminogen activator urokinase in urine from human fetuses and new-born (22) in spite of the high glomerular filtration rate and small power of reabsorption in the fetal kidney (26). If the production of urokinase is based upon the content of tissue activator in the kidney and involved in the general fibrinolytic activity in blood (10, 12, 15 17 29) the large activity in fetal renal parenchyme may contribute to the spontaneous fibrinolytic activity and very small plasminogen content in blood from especially immature but also mature infants as compared to adults (3 9 11 13 14 16 20, 30, 32).

SUMMARY

The concentration of plasminogen activator in the various organs of the huma genito-urinary tract and in the adrenals was in estigated in 15 fetuses (aged 15 to 24 weeks of pregnancy) nd in 7 premature or still-born infants (aged 26 to 38 weeks).

Cor ... to adult tissues the fetal ... showed ... fibrinoly ... acti ... both in ... and med ... a The activit ... o ... ves and uret... creased d... fetal life ... that of th... organism, whereas the fe ... ller ... a less active but with the ... mucosa and musculature ... active.

The activ ty of th ... reached the large ... whereas the fetal ... active as in adults, ... active than the a lu...

The fetal adrenal ... the adult one.

The cause and ... of the difference ... f activity which is pr... cussed.

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TEMPORARY MONOSACCHARIDE INTOLERANCE

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Failure to absorb the monosaccharides galactose and glucose has been reported by several authors (1 5 9) and the patients were successfully treated by using fructose as the sole dietary sugar Recently Burke & Danks (2) reported malabsorption of fructose as well as galactose and glucose in four infants in whom diarrhoea began between one and seven days following birth. Three of the babies recovered their ability to absorb these sugars between four and nine weeks after birth but this was delayed in the fourth child until the age of five months. The authors suggested that this temporary sugar intolerance might have been secondary to infection of the gastro-intestinal tract.

The purpose of this paper is to report further instance of intolerance of all monosaccharides including fructose and to discuss its possible association with staphylococcal infection. The dietary management of infants with sugar intolerance is described in detail.

CASE REPORT

Following a normal pregnancy and hospital delivery J V was the first born of young healthy West Indian parents. He weighed 3 4 kg at birth, was breast fed for 6 weeks and was described as a hungry baby who from birth passed 4–8 loose watery stools per day At the age of 3 weeks he began to vomit intermittently and despite changes in cow s milk preparations, the diarrhoea persisted. He was admitted to another hospital at 8 weeks of age, at which time he weighed 3 7 kg.

On National dried milk feeds he continued to have diarrhoea with blood in the stool 5 0) and intravenous fluids became ne correct severe dehydration A diagnosis of charide intolerance was considered and l free milk was given with some initial imp ment vitamin supplementation was given a ketovite tablets and liquid (see appendix) Desp te some initial improvement, diarrhoea recurred, and the diagnosis of ulcerative colitis was consider d and he was therefore given cortisol The fee were now changed to a milk formula containing glucose as the only carbohydrate but in sp te f this, severe diarrhoea persisted and t venous fluids were again necessary Whilst ec ng glucose, stools and urine gave a posit test for glucose with Clinistix (Ames & Co). Fructose was substituted for glucose but symptoms pers ted and intravenous fluids were once again necessary No pathogens were cultured from the stools and a seven-day course of neomycin did not result any improvement. He was then given a synthetic diet containing no carbohydrate and improvement was noted. Three weeks after admission he began to bleed from the nose, gastro-intestinal tract and several injection sites. His prothrombin time was prolonged and the bleeding stopped after giving intravenous vitamin K.

As this stage when 3 months old he was transferred to the Hospital for Sick Children. He was wasted (weight 3 8 kg) dehydrated and hungry The abdomen was tense and distended, the liver was of normal size and consistency He was febrile had a cough and rib recession and within 48 hours there was clinical and radiological evidence of a right upper lobe pneumonia (Fig. 1). At the time of his admission he was receiving 200 mg of hydrocortisone daily This was slowly reduced and subsequently stopped seven later

National dried milks are powdered milks, which when reconstituted have similar composition to cow milk but with added vitamin D. They are available in two types: fullcream and half-cream (partly skimmed).



Fig 1 Consolidation right upper lobe with early cystic changes.

The peripheral blood showed a polymorphonuclear leucocytosis (total WBC 20 600 per mm³ neutrophils 74%) the blood culture was sterile and no pathogens were grown from a throat swab. The BUN was 51 mg 100 ml and total serum proteins were 7.9 g 100 ml electrophoretic studies showed some increase in alpha 2 and gammaglobulin with some reduction of albumin. The results of other investigations were essentially normal and included haemoglobin, platelets, ESR, plasma electrolytes, plasma calcium and inorganic phosphorous, serum cholesterol alkaline phosphatase immunoglobulins, prothrombin and bleeding times, serum folic acid and vitamin B_{12}, urine examination including amino-acid pattern. Heaf test, sweat sodium and chloride concentration. No parasites or bacterial pathogens were isolated from repeated stool examinations.

The pneumonia was treated with intravenous cloxacillin and ampicillin and for the first 24 hours he was fed with a mixture of fructose, homogenised beef and arachis oil emulsion (prosparol). In view of his critical condition, formal carbohydrate load tests were considered unjustified. Severe diarrhoea persisted and a typical stool contained 500 mg/100 ml of fructose. He was then given lactase-treated breast milk for 5 days without improvement and during this time intravenous fluids were necessary to correct dehydration. Stool testing was positive for reducing substance with "Clinitest reagent tabs" (Ames & Co) and some stools were slightly acid (pH 6 0)

The infant therefore appeared to be intolerant of fructose glucose and galactose and it was decided to withhold all forms of sugar from the diet. At this stage blood pH was 7 47 standard bicarbonate 24.5 mEq/l and blood sugar 83 mg/100 ml. Initially the feed constituents per day were as follows 100 g homogenised beef (105 calories), 5 ml prosparol (70 calories), mineral and vitamins (see appendix)

There followed an immediate improvement in the consistency and frequency of the stools although they remained a little loose. The beef and prosparol were slowly increased by increments of 15 g of beef and 1 0 ml of prosparol to a total of 250 g of beef and 16 ml of prosparol. The weight loss which occurred during this period (see Fig. 2) can be attributed to the inadequate calorie intake which he received.

Medium-chain triglycerides are more easily absorbed from the gastro-intestinal tract than long-chain triglycerides and it was decided to introduce this fat into the diet as an attempt to further improve the consistency of his stools and general nutritional status. Two and a half millilitres per day of medium-chain triglyceride oil were given as a 50% emulsion (v/v) in water (see appendix) and substituted for an equal volume of prosparol and then slowly increased to 22.5 ml per day so as to completely replace the prosparol. This was followed by a further improvement in the consistency and frequency of the stools but no significant weight gain. During the time that he received the carbohydrate-free diet blood pH estimations were performed twice weekly and the urine was tested for ketones every day. Slight ketonuria was frequently observed but acidosis did not occur until 34 days after starting the carbohydrate-free diet when blood pH was 7.3 and standard bicarbonate 10.3 mEq/l. The blood sugar remained normal throughout this period. In view of the acidosis, 40 g of fructose as a 20% solution was given intravenously daily for 6 days without otherwise altering the diet. The acidosis rapidly subsided and his weight began to rise. He was now 4 1/2 months old, weighed 3.6 kg and was receiving 445 calories per day in the form of 250 g homogenised beef (265 calories) and

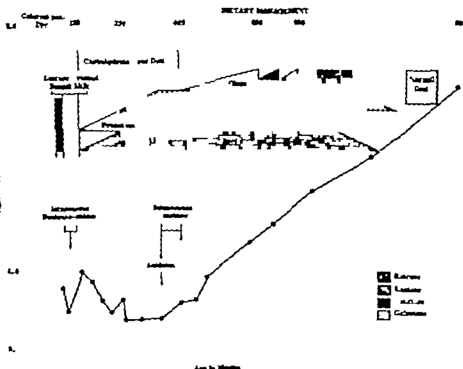


Fig ...3 V Dietary management.

...5 ml of medium-chain triglyceride oil (180 calories)

The pneumonia which was present on admission responded to antibiotic treatment and serial radiological examination of the chest showed gradual resolution accompanied by cystic changes (Fig. 3).

At this stage it was decided gradually to introduce oral glucose At first, he was given a total of 5 g glucose daily as a $^{1}/_{2}$% solution. The strength and volume of the glucose solution was slowly increased over the next month until he was receiving a daily intake of 48 g given as 4% solution. Throughout this period, the weight steadily rose, the stools remained free of glucose and the calorie intake had been increased to 650 calories per day

It was now clear that he could tolerate glucose and tolerance for the other 2 monosaccharides was investigated by first replacing 10 g of dietary glucose by fructose, and as this was well tolerated, half the dietary glucose (i.e. ...4 g) was replaced by fructose for week without fructose appearing in the stool The same regime was repeated using galactose instead of fructose and this sugar was also well tolerated and no galactose was detected in the stools. Tolerance for the disaccharides was then tested by adding 1 g of sucrose to the diet daily for a week, and when this caused no upset, lactose tolerance was tested by giving him fresh milk and this also proved to be well



Fig 3 Consolidation replaced by extensive cyst f

tolerated. The child was discharged on a normal diet when 8 months old and weighing 6.3 kg. His condition remained satisfactory and by 10 months of age he weighed 8 6 kg.

DISCUSSION

It is thought that the passage of galactose and glucose through the cell membrane is dependent on the presence of sodium, but does not require energy· within the cell these sugars are concentrated against a gradient and this process is both sodium- and energy-dependent (4) The absorption of fructose appears to be different· most of the fructose is absorbed by diffusion and passes to the liver for conversion to glucose but a small proportion is thought to be converted to glucose at the level of the intestinal cell (12) and such glucose is then absorbed in the manner described for that sugar A defect in the absorption of a sugar leads to its accumulation within the gut lumen and causes an osmotic diarrhoea with excessive bacterial fermentation and the passage of loose, watery acid stools. The reason for failure to absorb glucose, galactose and fructose is not as yet understood. Failure to absorb glucose and galactose may be familial (6) Three of Burke & Danks (2) patients with malabsorption of fructose glucose and galactose had normal jejunal mucosa. In two of the babies the history was very suggestive of a bowel infection having initiated the disturbance, and in one, Esch. coli (not typed) was isolated from the stools at the onset of symptoms. This, and the fact that all four babies recovered their capacity to absorb monosaccharides led the authors to conclude that the condition was secondary They were not able to show a family history of the condition. Our patient was thought to be too ill to justify biopsy We were unable to obtain a history from the family that a similar condition had occurred in relatives.

It is possible that in our patient a staphylococcal infection may have occurred soon after birth, resulting in diarrhoea and at a later date pneumonia (8, 10). Our patient probably regained monosaccharide tolerance at about the same time as the pneumonia resolved.

Mann *et al* (7) describe a four-month-old infant who developed pneumonia and probable staphylococcal septicaemia associated with disaccharide intolerance. When the pneumonia had been treated he regained tolerance for the sugars. Recently an infant aged 8 months was admitted to this hospital with disaccharide intolerance Four weeks after the onset of diarrhoea, she developed a lung abcess and when this had been treated, she became tolerant of the offending sugars. Clayton *et al.* (3) describe a twelve-month-old infant with staphylococcal septicaemia and disaccharide intolerance. Following recovery from the infection he was once again able to tolerate these sugars. As in our patient, all these infants had a staphylococcal infection associated with transient sugar intolerance and this association may reflect a particular susceptibility of the intestinal mucosa to this organism.

During the past year a number of infants, admitted to this hospital with severe gastro-intestinal symptoms due to sugar intolerance, have been treated in the first instance with carbohydrate free diets. This type of diet is a synthetic and unphysiological preparation and certain complications such as acidosis and hypoglycaemia might be expected to occur during such a feeding regime. For these reasons, such diets are not continued for longer than a few days, unless a sugar is being given intravenously or the circumstances are exceptional. In either instance, frequent blood sugars and pH estimations should be performed so that acidosis or hypoglycaemia may be detected and corrected at an early stage. It is worthy of note that neither acidosis or hypoglycaemia developed in our patient until such a diet had been given for nearly five weeks, although it was realised that sooner or later it was bound to occur and the acidosis was rapidly corrected with intravenous fructose. Another infant admitted to this hospital was intolerant of galactose and glucose but did not develop acidosis until a similar diet had been continued for two weeks, however it is to be anticipated that in the majority of cases the onset of acidosis would be much sooner and in one infant occurred after 48 hours. Severe symptoms caused by hypoglycaemia occurred in one patient after such a diet had been given for only 4 days.

The case also illustrates the importance of adequate vitamin supplementation when special diets are being given (7). A bleeding tendency in the noted was observed with a prolonged prothrombin time and was promptly corrected with vitamin K. At this time his diet was supplemented

with ketovite (see appendix) which does not at present contain vitamin B. Burke & Danks (2) describe apnoeic attacks associated with a red raw mucosa on the tongue and in the mouth and rectum in one of their cases the infant was found to be receiving inadequate quantities of vitamin B and correction of this led to dramatic improvement.

The study of sugar tolerance by giving one single large dose of sugar may have serious consequences if the infant is already seriously ill and is in fact intolerant. In such cases we have found it advantageous to distribute the same quantity of sugar over a 24-hour period, judging its effects by the character of the stools and by chromatographic estimation of the amount of sugar passed in stools and urine.

SUMMARY

An infant presented at three months with intolerance of glucose, galactose and fructose. It was thought that this was possibly secondary to staphylococcal infection. Recovery of monosaccharide intolerance coincided with recovery from infection. The infant was treated with a sugar-free diet which is described in detail. It is important to detect and correct ketosis and/or hypoglycaemia at an early stage when such a diet is used.

ACKNOWLEDGEMENTS

Dr Jim Appleyard gave helpful information regarding the sequence of events which occurred prior to the infant' admission to the Hospital for Sick Children.

M.C.T. oil was kindly supplied by the Mead Johnson Research Centre. The survival and subsequent progress of J. V. was largely due to the expert nursing care which he received from Sister Margaret Leaversley and her staff.

APPENDIX

This appendix is intended as a guide to the dietary control of sugar intolerance in those infants who have failed to improve on the special proprietary preparations which are now available for the treatment of this condition. The diet, outlined below, is indicated in only a small number of infants with this condition, and its use should be restricted to these children.

Preparations Used

(a) *Homogenised beef*

Blue Label or Star Brand Kosher Beef in beef broth with salt. Composition (carbohydrate-free): 15% protein, 5% fat, 105 calories per 100 g. calcium 6 mg. phosphorus 95 mg, sodium 80 mg, and potassium 140 mg per 100 g. *Kosher Meat Canning Co. Ltd., Winnipeg, Canada.* Available at Jewish retail shops. This particular type of homogenised beef is finely ground which, when diluted with water is suitable for feeding through a large-holed infant feeding bottle.

Most standard hospital and household homogenisers result in a fibrous meat homogenate which is only suitable for spoon feeding. In this case, rabbit and chicken breast are the most suitable sources of protein due to their fine fibre.

(b) *Prosparol*

50% arachis oil. Duncan Flockart Ltd., London E2. (Prosparol is an arachis oil emulsion of long chain triglycerides.)

(c) *Medium chain triglyceride oil*

Product 7010X. Mead Johnson Research Centre, Evansville, Indiana, USA. MCT emulsion is prepared by emulsifying MCT oil with an equal volume of water and gum acacia is used as the emulsifying agent. This may be prepared in an electric homogeniser using the following proportions:

300 ml MCT oil, 300 ml water 30 g gum acacia.

The resultant mixture keeps relatively well, if refrigerated, but if long storage is desired, a preservative such as chloroform water should be used to prevent mould growth. MCT should be used with caution as it may cause colicky abdominal pain and vomiting when first introduced into the diet especially if this is done too rapidly or if the MCT is in a too-concentrated form.

N.B. "Portagen" is a milk formula, made by Mead Johnson in which the fat content is in the form of MCT. It contains lactose and sucrose. MCT oil will be available in England in the near future.

(d) *Mineral supplements*

Synthetic feeds require a complete mineral supplement including the trace elements. Such a mixture was devised by Westall (11) for use with synthetic amino acid diets and has been adopted for the dietary control of children with sugar intolerance at this hospital. *Half the recommended quantities are usually sufficient for the infant.*

Complete mineral supplement (11)

Mineral mixture A	Daily requirement
Calcium lactate	4.5 g
Calcium chloride ($CaCl_2$ $2H_2O$)	0.3 g
Dipotassium hydrogen phosphate	1.5 g
Disodium hydrogen phosphate	1.0 g
Magnesium sulphate	0.8 g
Ferrous sulphate	20 mg
Copper sulphate	2 mg
Zinc chloride	2 mg
Manganese sulphate	2 mg
Potassium iodide	80 µg
Potash alum	30 µg
Cobalt sulphate	30 µ
Sodium molybdate	3
Total	

Mineral mixture B

Anhydrous sodium carbonate	0 17 g
Potassium carbonate	0.22 g
Calcium carbonate	0.31 g
Total	0.7 g

Elemental composition of complete mineral A and B

	Daily
	397.8 mg
Calcium	758.8 mg
Potassium	1975 mg
Chlor	959 mg
Phosphorus	485 mg
Magnesium	78.9 mg
Iron	4.0 mg
Copper	0.51 mg
Zinc	0.96 mg
Manganese	0 49 mg
Iodine	61 µg
Aluminium	1 1 µg
Molybdenum	119 g

The c' ments included in the above mineral mixtu sen ial f long term treatment is necessary

( ) 1 ns

All synthe diets should be supplemented with complete tamin preparation Most of the common preparations do not contain the "minor itamins such as choline chloride calcium pantothenate, inositol, biotin, alpha-tocopherol, B_{12} and folic acid, and deficiency states may occur (7) Ketovite tablets (dosage 1 t.d.s.) plus ketovite liquid (5 ml daily) by Paines & Byrne Ltd contain adequate amounts of the vitamins required by man except vitamin K. A can be seen from the composition, both the tablets and the liquid are required and the one is not substitute for the other

Composition

Ketovite tablets (contents per tablet)

	mg
Aneurine hydrochloride	1.0
Riboflavine	1.0
Pyridoxine hydrochloride	0 33
Nicotinamide	3.3
Calcium pantothenate	1 16
Ascorbic acid	16 6
α-Tocopheryl acetate	5.0
Inositol	50 0
Biotin	0 17
Folic acid	0 25

Ketovite (supplement) Liquid contains in each 5 ml dose

Vitamin A	2,500 units
Vitamin D	400 units
Choline chloride	150 mg
Cyanocobalamin	12.5 g

Both preparations are lactose and sucrose free Vitamin K (Acetomenopthone 0 5 mg per tablet) ill be included in ketovite tablets in the near future

The Dietary Regime

The dietary control of sugar intolerance has been successfully achieved in a number of infants by using synthetic diets composed of the above-mentioned preparations when proprietary products such as one of the Galactomins (Trufood Ltd) have not proved adequate Unless there is a contraindication, glucose or fructose (5–10% solution) is included in the feeds after the fourth day so as to prevent ketosis and hypoglycaemia developing, and to increase the calorie intake of the infant. The feeds are calculated for the individual and to begin ith are given as a small volume frequent feeding schedule e.g. 1 or 2 hourly Despite the low calorie intake, the initial feed has been found to be most successful hen it is given as a very dilute solution. The feed is calculated as follows: / oz (7 g) homogenised beef/kg body wt/day is diluted with the volume of water required by the infant as fluid intake. The fluid intake will, of course, vary with the degree of dehydration present in the infant. This provides 1 g of protein/kg body wt/day If the beef is tolerated, it is increased every 1–2 days by / oz (7 g) homogenised beef/kg body t/day until 1 1 / oz (28–42 g) of homogenised beef/kg body t/day are being given which provides 4.3–6.4 g of protein/kg body wt/day Mineral mixture A and B (/ quantity) is added to the feed but the recommended dose of ketovite tablets and liquids are given separately to the feeds.

Prosparol or MCT emulsion is added to the feed so as to provide extra calories. Initially 5 ml of emulsion per litre of the total volume is added and this is then increased by 1 2 ml daily until a total of 45 ml/l is being given. The fat content at this stage is equivalent to that in half-cream milk.

For reasons already stated glucose or fructose given in small dilute quantity is added to one feed only after the fourth day (Glucose is readily available and inexpensive and therefore would seem preferable to fructose) This is added t the feed at concentration of 0.5% and if tolerated is gradually added t the other feeds over the next 3–4 days. The carbohydrate content of the feeds is then slowly increased by increments of 0.5 until a solution of 5–10% carbohydrate is tolerated (most infant feeds contain 7% carbohydrate). If the initial introduction of glucose or fructose is not tolerated the carbohydrate-free diet is supplemented ith intravenous carbohydrate and the patient challenged at later date with one or other of the above monosaccharides although in our experience this has not usually been necessary

Despite the initial low calorie intake the fat and protein constituents of the diet should be increased slowly and the cautious reintroduction of carbohydrate into the feeds after the fourth day should be as dilute glucose or fructose solution, and then slowly increased by altering the concentration of the sugar solution.

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BLOOD GLUCOSE OF THE HUMAN FETUS PRIOR TO AND DURING LABOR

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Neonatal hypoglycemia offers a good example of a postnatal disorder which is obviously influenced by conditions prevailing *in utero* This is evidenced by the frequent occurrence of toxemia as a predisposing factor in low birth weight neonates with hypoglycemia, and by the association of maternal hyperglycemia and neonatal hyperinsulinism, a postulated sequence in infants of diabetic mothers (for review see ref 3). With these considerations in mind it would obviously be interesting to obtain reliable information on the intrauterine blood glucose levels of the human fetus.

Animal experiments (7 18) and studies on maternal and fetal blood glucose concentrations at the time of birth (10 12, 21) have indicated that the fetal levels are constantly somewhat lower than the maternal ones, and that a process of facilitated diffusion is evidently responsible for the placental transport of glucose (23). In the course of delivery the blood glucose of the mother is known to rise significantly (9) and a comparable elevation was suggested to occur in the fetus on the basis of comparison of umbilical blood glucose values after labors of varying duration (9) This assumption has recently been verified by direct sampling of fetal capillary blood during delivery (14 17), made possible by the technique introduced by Saling (16) However even the possibilities offered by this method have not yet been employed to study the basal situation, i.e to measure fetal capillary blood glucose before the onset of labor

We have investigated the blood glucose values of human fetuses prior to and during labor after both normal and abnormal pregnancies.

PATIENTS AND METHODS

Patients

The investigations were performed in 38 cases, in which labor was induced for reasons to be mentioned. The induction was accomplished by discision of the membranes, and the first samples of fetal blood were obtained at the same occasion, prior to the onset of uterine contractions. In five additional cases the first sample was taken after the spontaneous onset of labor

The cases were divided into four groups.

I *Normal.* As some indication for the induction of labor was present in all cases, none could be classified as strictly uneventful. However the following 12 cases with minor abnormalities were considered to be normal with regard to carbohydrate metabolism: 8 cases with suspected erythroblastosis (all with an umbilical blood hemoglobin concentration of 14 0 g 100 ml or more and a reticulocyte count of 5.0% or less), 2 cases with signs of mild hepatic involvement in the mother (itching, elevated serum GOT and GPT values) and one case each of irregular heart sounds and twin pregnancy Further details of pregnancy and delivery are depicted in Table 1 All the infants were *born in good condition; the Apgar score was 9 or 10 at* the age of five minutes in each case. Mild and transient fetal acidosis was observed in two instances.

II *Toxemia.* Diastolic blood pressure of 90 mmHg or more and/or albuminuria were accepted as criteria for toxemia. Eight such cases were studied prior to and three additional ones during labor (Table 1). The Apgar score at five minutes was 8 or more in all infants, and two cases of mild fetal acidosis were observed. T of the infants had birth eight below the 10th percentile for gestation, and one of these developed symptomatic hypoglycemia during the second day of lif All the others had an uneventful neonatal course.

III. *Rh-immunization* I the nine cases of this group the umbilical blood hemoglobin concentration was less *than 14.0 g/100 ml and the reticulocyte count more than* 5.0% (for additional information see Table 1) In each case the acid-base values remained normal during labor and all infants received an Apgar score of 8 or more at five minutes. One or more exchange transfusions were performed for every infant by the age of 48 hours

Table 1 *Clinical data on the cases*

	Normal	Toxemia	Rh-immunization	Prolonged pregnancy
No. studied before labor	12	8	9	8
Total no. studied	12	11	9	10
Primiparous	3	7	—	7
Multiparous	9	4	9	3
Gestation, days				
Mean	272	276	265	291
Range	264–290	55–299	261 269	285–296
Birth weight, g				
Mean	3,363	2,845	3,230	3 632
Range	2340–4380	1890–4580	2570–3590	3300–4070
Complications of delivery	1 Cesarean section 1 Vacuum extraction 1 T ns	1 Vacuum extraction 1 Triplets	—	1 Cesarean section 2 Vacuum extraction

IV *Prolonged pregnancy* Duration of pregnancy for 10 or more days after the expected date and/or positive radiological evidence (placental calcification and/or advanced fetal skeletal age) were considered as criteria for this group of ten cases. Cesarean section was performed in one case because of cervical dystocia associated with fetal acidosis (pH 7.26, standard bicarbonate 15.8 mEq/l), but no other acid-base disturbances were observed. The Apgar score was 8 or more in all cases at five minutes. One infant was noted to have cleft lip and esophageal atresia, and was operated at the age of 12 hours. For additional information see Table 1

Methods of sampling

Fetal blood, obtained by pricking the hyperemic scalp, was sucked into long glass capillary tube containing heparinized thread. Aliquots of the contents of the capillary ere immediately precipitated in triplicate with sodium hydroxide and zinc sulphate. Maternal capillary blood was simultaneously collected from fingertip and treated similarly

After the basal values prior to the onset of labor had been obtained, further samples ere taken at intervals as labor progressed. In 32 cases the umbilical cord was double clamped immediately after delivery before the first breath, and samples of arterial and venous cord blood ere separately precipitated.

Chemical methods

The concentration of glucose in the supernatant fraction of the precipitated samples as determined with specific enzymatic method (11), using glucose oxidase reagent obtained from AB Kabi, Stockholm. The parameters of acid-base balance in blood were determined with the method of Astrup *et al.* (1), using an apparatus supplied by Radiometer Copenhagen.

RESULTS

Assessment of the value of capillary blood glucose determinations

We have tried to assess the value of information obtained from the scalp capillary samples by com parison of the glucose levels with those measured in the umbilical vessels. Of course, such a com-

Table 2. *Comparison of the glucose concentration in fetal scalp capillary blood, obtained immediately before birth, to the values in the umbilical artery (UA) and vein (UV)*

Fetal capillary blood mg per 100 ml	Min before birth	UA (mg per 100 ml)	UV (mg per 100 ml)	Difference (capillary/UA) mg per 100 ml	% of UA
83	1	79	89	+4	5
74	1	70	84	+4	6
64	3	63	71	+1	2
80	4	74	84	+6	8
69	5	62	92	+7	11
67	6	62	77	+5	8
75	6	84	92	−9	11
61	7	70	86	−9	13
75	10	78	91	−3	4
Mean 72		71	85	.3ª	7.6

Mean bsolute capillary/UA difference (mg per 100 ml). 5ª

Table 3 *Maternal and fetal capillary blood glucose levels and materno-fetal gradients before the onset of labor in normal cases and in cases complicated by toxemia Rh-immunization or prolonged pregnancy*
Results are the means ± standard deviations

Group	No. of deter minations	Maternal glucose (mg/100 ml)	Fetal glucose (mg/100 ml)	Gradient (mg/100 ml)
Normal	12	89 ± 14	64 ± 10	25
Toxemia	12	91 ± 16	61 ± 8	30
Rh-immunization	1	90 ± 17	83 ± 13	7
Prolonged pregnancy	*10*	*76 ± 10*	*58 ± 6*	*18*

parison is feasible only in cases, in which the time interval between the samples is so short that fluctuations in either direction are unlikely to have occurred.

Table 2 summarizes nine cases, in which the last capillary sample was taken ten minutes or less before the clamping of the cord. The dif ferences between the capillary and umbilical ar tery values were generally much smaller (mean 7 6%) than those between the capillary and umbilical vein values (mean 16.3%) In six cases the capillary value was between the corresponding values in the umbilical artery and vein, while in three cases it was somewhat lower than either of these. Thus the concentration of glucose in scalp capillary blood can be said to reflect that pre vailing in the fetal arterial system, and thus give indication of the supply of glucose to the fetal tissues. Similar conclusions have been reached concerning the acid-base values in scalp capillary blood (2..).

Fetal blood glucose levels prior to the onset of labor

The results of the measurements of fetal blood glucose levels before the first uterine contractions are shown in Table 3 The mean value for all the cases studied was 61.5 mg/100 ml, and the variations within, and also between, the four groups was relatively small and without statistical significance. Corresponding maternal glucose values also agree closely within the groups, the mean in mothers with prolonged pregnancy being, how ever somewhat lower than in the others. This is reflected in the somewhat lower materno-fetal gradient.

Changes during labor and delivery

Correlation of the rise in maternal and fetal blood glucose with the progress of labor is complicated by the lack of satisfactory criteria for the comparison of this progress between cases. As the quality of labor pains is quite variable their dura-

Table 4 *Fetal and maternal blood glucose values during labor and at the time of birth*
Cervical dilatation and time f sampling before birth are shown as indices of the progress of labor

	Cervical dilatation, cm					Time before birth, min		
	1 2	3–4	5–6	7–8	9–10	Over 360	300–360	240–299
Fetal glucose								
Mean	62	60	70	66	73	61	67	67
Range	30–79	41–87	58–94	53–75	50–103	43–85	54–87	52 75
No. of determinations	27	30	10	7	21	35	11	7
Maternal glucose								
Mean	88	83	102	98	106	87	96	95
Range	61 117	56–105	76–138	84–110	80–130	56–127	76–115	81 110
No. of determinations	[illegible]	29	12	7	12	34	8	7

Fetal and maternal glucose at birth

	Umbilical artery	Umbilical vein	Mother
Mean	68	78	110
Range	36–109	56–120	80–149
No. of determinations	31	32	19

tion cannot be a solid basis for reference. Similar variability concerns the dilatation of the cervix. However as no better criteria were available for comparative purposes, the data on all the cases studied were combined and grouped according to cervical dilatation as well as time of sampling, which is expressed as minutes before birth (Table 4).

In the early part of the first stage of labor the fetal blood glucose tends to remain at the *ante partum* levels or even decrease slightly The rise towards values observed in umbilical blood occurs, for the most part, during the last hour of labor or at the cervical dilatation of 9–10 cm. The same, but quantitatively more marked, trend can be discerned in the maternal concentrations. From the beginning to the end of labor as determined by cervical dilatation, the increase in both fetal and material blood glucose is statistically significant (p less than 0 01 for both) However individual variations are obviously large.

As mentioned above, one infant of a toxemic mother developed symptomatic hypoglycemia in the neonatal period. Unfortunately fetal blood glucose was not measured before the onset of labor in this case, but two values obtained during labor 65 and 67 mg/100 ml, were normal, whereas the umbilical artery level was relatively low 44 mg/100 ml. The lowest concentration in the whole series, 36 mg/100 ml was measured in the umbilical artery of the secondborn twin of

180–239	120–179	60–119	30–59	0–29
61	63	67	77	69
51 79	41–90	55–97	62 103	50–89
9	9	10	4	18
82	96	92	114	103
64–99	80–127	76–138	88–129	84–130
10	8	9	4	10

a normal mother but in this case no postnatal problems were encountered.

Comparison of the changes in blood glucose during labor or of the umbilical values did not reveal any significant differences between the four groups of cases studied. Neither could the glucose levels be correlated with changes in fetal acid-base values in the few cases, in which such changes were observed.

DISCUSSION

In several animal species, for instance in sheep, Rhesus monkey and rabbit, the blood glucose of the fetus remains at approximately half the maternal concentration up to delivery (18) at which time the associated stress and hypoxia presumably bring about an increase to the levels observed postnatally (5) Analogous suggestions ha e been made concerning man (4 19)

The technique of fetal capillary blood sampling (16) has been employed in some studies on the blood glucose values of the human fetus during labor Schönfelder (17) demonstrated a progressive rise in fetal values towards delivery generally in good correlation with the maternal levels, but the presence or absence of uterine contractions at the time of the first samples was not stated. The close relation of the changes in maternal and fetal glucose concentrations was confirmed by Paterson *et al.* (14), who also commented on the increase in the levels during labor

W are not aware of any reported studies on the blood glucose values of the human fetus *prior* to the onset of labor except in one case (15) in which the conditions could hardly have been called physiological Therefore, we can compare our data only with results obtained under somewhat different or undefined circumstances. The mean value measured by Schönfelder (17) in the early part of the first stage of labor was 51 8 mg 100 ml or somewhat lower than our mean basal level, and the mean rise during labor was considerably greater in his series than in ours. The mean fetal level observed by Paterson *et al.* (14) was 74 mg/100 ml, but this was measured at an undefined stage of labor Our data on umbilical arterial and venous blood glucose are in agreement with both these reports.

Toxemia of pregnancy is considered to be an important cause f placental insufficiency (13),

and is associated with the birth of "small for dates" infants and a high incidence of neonatal hypoglycemia (3). Placental function is liable to suffer also in prolonged pregnancy (20), although neonatal problems of blood glucose regulation are rare (2). Increased pancreatic insulin content (6) and hypoglycemia (8) have also been documented in association with erythroblastosis. Surprisingly none of these factors seemed to influence the fetal blood glucose levels in any way either *in utero* or during the stress of delivery It can be assumed that if the placenta plays a role in creating the capability to maintain normal blood glucose levels during the neonatal period, inadequate performance of this role does not markedly involve the transport of glucose itself, and thus is not readily reflected in the fetal blood glucose levels.

SUMMARY

With the technique of scalp capillary blood sampling, the blood glucose levels of human fetuses were studied in normal cases as well as in cases with toxemia, Rh-immunization or prolonged pregnancy Before the onset of labor the mean level was 61.5 mg/100 ml, and an increase was demonstrated as labor progressed. The maternal concentration was always somewhat higher than that in the fetus, and an analogous increase was observed during labor No significant differences could be demonstrated between the normal and abnormal cases, either prior to or during labor

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NEONATAL HYPOGLYCEMIA

I Occurrence of Hypoglycemia in Patients with Various Neonatal Disorders

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Recognition of neonatal hypoglycemia as a potential cause of cerebral damage (1 4 12) has emphasized the importance of early detection and treatment of the disorder The blood sugar levels of newborn (6, 9), particularly premature infants (3 22) are known to be low by adult standards, but the methodological sources of error in the blood glucose measurements, caused by accelerated glycolysis (3) and interfering substances (11), have only recently been clarified. Screening programs for hypoglycemia, using adequate methods for true blood glucose determinations, have so far been restricted to low birth weight infants (5 17), infants of diabetic mothers (14) and twins (20). Reports on the association of hypoglycemia with other neonatal disorders have been more or less incidental.

In September 1965 a program for the investigation of problems associated with neonatal hypoglycemia was initiated in the Children's Hospital of the University of Helsinki. The purpose of the first part of the study was to find out the conditions predisposing to or associated with, hypoglycemia. In this communication, we describe the procedures and results of blood glucose determinations in an extensive series of newborns, and present an estimation of the incidence of neonatal hypoglycemia in high-risk groups.

PATIENTS AND METHODS

Patients

Most of our neonatal patients come from two large obstetric hospitals in Helsinki, the Department of Obstetrics and Gynecology of the University Central Hospital and the Midwifery Institute, with a total of approximately 13,000 births per year. In addition, infants with more severe disorders are referred from regional obstetric and pediatric hospitals. Due to special arrangements, the majority of infants of diabetic, Rh-immunized and severely toxemic mothers are born at the Department of Obstetrics and Gynecology of the University Central Hospital, and are routinely transferred to our care.

Between September 1st, 1965, and December 31st, 1966, a total of 1293 infants were admitted to the newborn wards of the hospital. In 964 of these two or more blood glucose determinations were obtained before the age of five days, and these will be considered in this report. Their clinical features are summarized in Table 1

In 301 cases the birth weight was 2500 g or less. The number of infants with birth weight below the 10th percentile for gestation (assessed using unpublished intrauterine growth curves based on a Finnish obstetric population) was 121 These infants will be referred to as "dysmature" whereas the term "premature" will be reserved for infants weighing below 2500 g at birth, but with proportionately short period of gestation. A slight male predominance (1.2 1) was noted among the dysmature infants, and maternal toxemia (44 cases) and erythroblastosis (22 cases) were the most common associated disorders.

The 30 pairs of twins studied included 8 discordant ones (2), in which the smaller malnourished member was in many ways comparable to a dysmature infant. The possibility of discordance existed in two additional cases, in which the birth weight of only one member was known.

Maternal diabetes or prediabetes had been diagnosed in [illegible] of the 75 cases, in which the birth weight of the infant exceeded 4000 g

Eighty infants (8.3%, of the series) died during the first ten days of life, and they were in a more or less moribund condition at the time of the blood glucose determinations. Hypoglycemia in these cases will be referred to as "terminal" The principal causes of death were cerebral hemorrhage, alone (8 cases) or in combination with pulmonary disorders (13 cases), and isolated respiratory difficulties (28 cases)

The causes of admission are shown to illustrate the wide variety of disorders treated They represent the points of view of the referring physicians, not our final diagnoses.

Almost a fourth of the patients received intravenous glucose at some time during the first week of life, and in 41 of them no blood glucose determinations were performed prior to the start of the infusion.

Table 1. *Clinical data on 964 newborns screened for hypoglycemia*

Sex			
Males	507		
Females	457		
Birth weight, g			
Below 1250	40		
1250–2500	261		
2501–4000	588		
4001–4500	5		
Above 4500	23		
Dysmature	121		
Twin pregnancies			
Both members studied		30	
One member studied		20	
Total		50	
Discordant pairs		8 (27)	
Triplets		2	
Deaths (before 10 days of age)			80
Birth weight below 2500 g			52
Before 5 days of age			65
Causes of admission			
Maternal diabetes or prediabetes	80		
Asphyxia, respiratory distress	230		
Erythroblastosis	293		
Hyperbilirubinemia	52		
Cerebral damage	47		
Infection	19		
Heart disease	14		
Prematurity	1_0		
Hyperglycemia	20		
Miscellaneous	89		

Methods of screening and treatment

The first capillary sample was obtained within 8 hours of admission (and immediately in cases with suspicious symptoms), and the glucose levels were subsequently checked each morning until the infant was one week old. All values between 20 and 30 mg/100 ml were controlled at four-hourly intervals, until the blood glucose was stabilized above 30 mg/100 ml or depressed below 20 mg/100 ml.

If the result at any time was 20 mg/100 ml or less, a catheter was immediately passed into the umbilical vein, a blood sample was drawn and 1.0–1.5 grams of glucose were rapidly injected, after which an intravenous infusion of 10% glucose at the rate of 80–100 ml/kg body weight/day was started. The effect of the initial injection on symptoms was closely observed, but the procedure was identical in asymptomatic cases. During therapy as well as for two days after its cessation the blood glucose levels were monitored five times a day. The infusion was tapered and stopped after the levels remained above 40 mg/100 ml, but not until after a minimum of 24 hours. Peroral feeds consisting of 10% glucose and breast milk were started at the age of 12–36 hours, depending on the clinical condition of the infant. Hydrocortisone was administered to certain patients in a manner to be described in another report (18).

Chemical methods

The heel capillary blood samples were taken in duplicate and immediately precipitated with sodium hydroxide and zinc sulphate (21). The glucose concentration was measured with the glucose oxidase method (13), using the commercial reagent supplied by AB Kabi, Stockholm.

RESULTS

During the period of study an average of five blood glucose determinations per patient were performed. One or more values of 30 mg/100 ml or less were observed in 252 cases (26.1%). These observations are summarized in Table 2, arranged in the following five groups: I. Two or more values of 20 mg/100 ml or less. II. one value of 20 mg/100 ml or less and one or more of 21–30 mg/100 ml. III, two or more values of 21–30 mg/100 ml. IV. one value of 20 mg/100 ml or less, the others above 30 mg/100 ml. V. one value of 21–30 mg/100 ml, the others above 30 mg/100 ml. Although the significance of single pathological measurements is doubtful, groups IV and V are included for the sake of completeness. In the grouping of patients, factors known or claimed to be associated with hypoglycemia have been used as a basis. Infants meeting the criteria of two or more groups appear in the uppermost one in the left hand column of the table.

Dysmaturity, maternal diabetes and terminal hypoglycemia account for a total of 41 of the 55 cases, in which two or more blood glucose values of 20 mg/100 ml or less were measured (Group I). Two smaller members of discordant twin pairs are included in the dysmature group. Six of the 11 terminal cases had primary cerebral damage. In 9 male infants maternal toxemia was the only apparent predisposing factor, and 7 of these had a birth weight between the 10th and 50th percentiles for gestation. The sex ratio in Group I was 1.75 : 1 in favor of the males.

The predisposing factors and sex distribution in Group II resembled closely those of Group I, whereas in Group III the sex ratio was reversed and the number of premature infants exceeded that of dysmature ones.

The first blood sample was taken during the first day of life in 74 cases, during the second day in 114 cases and later in 108 cases. By the

Table 2. *Distribution of the observed low blood glucose values according to the diagnosis and sex of the infants*
M, males; F, females

	Low blood glucose values (for definition of groups see text)									
	I		II		III		IV		V	
Patient group	M	F	M	F	M	F	M	F	M	F
Terminal	6	5	4		2	—	—		5	3
Maternal diabetes	6	5	7		4	7	1		8	7
Dysmaturity	11	8	5	3	4	5	1	—	6	9
Maternal toxemia	9	—	—	—	2	3	1	3	—	3
Erythroblastosis		—	—	—		1	2		1	8
Prematurity	—	1	3	—		8	1			5
Asphyxia, RDS	1	1	—	—	2	—		—	4	4
Adrenal insufficiency	—	—	—	—	—	2	—	1	—	—
Others	—	—	—	—	1	2	—	1	3	5
	35	20	19	9	19	28	8	13	4	56
Total	53		28		47		21		101	
Per cent of patients	5.7		2.9		4.9		—		10.5	

age of 24 hours, the first low glucose value had been obtained in 46 infants of Group I, 27 infants of Group II and 33 infants of Group III. The majority of cases diagnosed after the first day of life were among those admitted later and only one infant of Group I and nine in Group III had been in the hospital for over 24 hours before the first low value was obtained.

The magnitude of the risk for hypoglycemia in the suggested "high-risk" groups has been estimated and is presented in Table 3. Infants receiving intravenous glucose without prior blood glucose determination have been excluded, with the exception of the terminal cases and RDS-infants, most of which had received small amounts of 10 % glucose in connection with bicarbonate administration. These exclusions and the contribution of a few cases to the figures of two "high-risk" groups account for the differences between Tables 2 and 3.

The incidence of Group I values was highest in dysmature infants (20 %), especially the males (25 %), and considerable also in infants of diabetic mothers (16 %) and terminal cases (15 %). On the other hand, hypoglycemia was rare in infants with low birth weight without dysmaturity (1.4 %) and in respiratory distress syndrome (1.9 %). Although severe erythroblastosis is evidently a significant predisposing factor for hypoglycemia (18), the low overall incidence in this series (1.4 %) is explained by the fact that even the mildest cases are included in the total of 285 patients with this disease. The majority of patients in the "Others" group were infants of toxemic mothers, but due to lack of detailed obstetric information on the whole population it

Table 3. *Incidence of low blood glucose values in certain groups of new born infants*

		Low blood glucose values (for definition of groups see text)					
		I		II		III	
Patient group	N of cases	No	%	No	%	No	%
Infants of diabetic mothers	73	1	16	9	12.3	32	43.8
Low birth weight infants							
Dysmature	104	1	20.2	9	8.7	43	41.3
Not dysmature	141	2	1.4	3	2.1	18	12.8
Terminal illness	80	1	1 0	7	8.8	20	25.0
Erythroblastosis	83	4	1.4	—	—		
Respiratory distress syndrome	53	1	1.9	—	—		
Others	188	10	5.3	1	0.5		

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Dysmature	121		
Twin pregnancies			
Both members studied		30	
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Birth weight below 2500 g			52
Before 5 days of age			65
Causes of admission			
Maternal diabetes or prediabetes		80	
Asphyxia, respiratory distress		230	
Erythroblastosis		293	
Hyperbilirubinemia		52	
Cerebral damage		47	
Infection		19	
Heart disease		14	
Prematurity		120	
Hypoglycemia		0	
Miscellaneous		89	

Methods of screening and treatment

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If the result at any time was 20 mg/100 ml or less, a catheter was immediately passed into the umbilical vein, a blood sample was drawn and 1.0–1.5 grams of glucose were rapidly injected, after which an intravenous infusion of 10% glucose at the rate of 80–100 ml/kg body weight/day was started. The effect of the initial injection on symptoms was closely observed, but the procedure was identical in asymptomatic cases. During therapy as well as for two days after its cessation the blood glucose levels were monitored five times a day. The infusion was tapered and stopped after the levels remained above 40 mg/100 ml, but not until after a minimum of 24 hours. Peroral feeds consisting of 10% glucose and breast milk were started at the age of 12–36 hours, depending on the clinical condition of the infant. Hydrocortisone was administered to certain patients in a manner to be described in another report (18).

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Dysmaturity, maternal diabetes and terminal hypoglycemia account for a total of 41 of the 55 cases, in which two or more blood glucose values of 20 mg/100 ml or less were measured (Group I). Two smaller members of discordant twin pairs are included in the dysmature group. Six of the 11 terminal cases had primary cerebral damage. In 9 male infants maternal toxemia was the only apparent predisposing factor, and 7 of these had a birth weight between the 10th and 50th percentiles for gestation. The sex ratio in Group I was 1.75 : 1 in favor of the males.

The predisposing factors and sex distribution in Group II resembled closely those of Group I, whereas in Group III the sex ratio was reversed and the number of premature infants exceeded that of dysmature ones.

The first blood sample was taken during the first day of life in 742 cases, during the second day in 114 cases and later in 108 cases. By the

and therapy is started, if one low value is obtained.

These procedures have been followed in our hospital since January 1st 1967. During the succeeding 8 months, 40 cases of significant hypoglycemia were detected. Ten of these were classified as terminal, 13 were infants of diabetic mothers and 2 had severe erythroblastosis, whereas the rest were idiopathic. If all the 95 cases of significant hypoglycemia seen in the course of two years are considered in retrospect, the screening procedure outlined above would have covered all but four of them. This fact attests to the "predictability" of the disorder and the feasibility of screening.

SUMMARY

Two or more serial blood glucose determinations were performed before the age of five days in 964 neonates treated in the Children's Hospital. Two or more values of 20 mg/100 ml or less, considered to indicate significant hypoglycemia, were observed in 55 infants, or 5.7% of those studied. The incidence of significant hypoglycemia was 20% in dysmature infants, 16% in infants of diabetic mothers and 15% in critically ill infants dying within ten days of birth. No other important predisposing factors were detected. A screening procedure for the early diagnosis of neonatal hypoglycemia is described.

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THROMBASTHENIA. A STUDY OF TWO SIBLINGS

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The group of congenital hemorrhagic disorders charactertzed by prolonged bleeding time and normal platelet count has been recently separated into three categories (3 19 26, 29): prolongation of bleeding time without platelet anomaly prolonged bleeding time associated with plasma and platelet defects (von Willebrand's disease), and true thrombocytopathy with qualitative platelet defect only The latter category has been further subdivided into two entities, that with normal clot retraction, now designated thrombocytopathy and that with disturbed clot retraction thrombasthenia or Glanzmann's disease (10). Recent reports (5 17) emphasize the abnormal adsorption properties of the platelets in thrombasthenia. Other studies (4 13 19) relate the platelet abnormality in thrombasthenia to a defect in the platelet membrane We present here studies performed on two siblings whose hemorrhagic disorder confirms the recently diagnostic criteria of thrombasthenia.

METHODS

Blood was collected by venipuncture, using siliconized glass ware. Anticoagulation was achieved hb 1 volume of 3.8% disodium citrate to 9 olumes of blood. Platelet-rich plasma (PRP) was prepared by centrifuging blood at 125 g for 10 minutes at 4°C. Platelets were isolated from PRP by centrifugation at 1085 g for 15 minutes at 4°C, and the sediment was thrice washed in Owren buffer Platelet-poor plasma (PPP) was prepared by centrifuging the blood at 1085 g for 30 minutes at 4°C. Routine coagulation tests such as clotting time, platelet count, prothrombin consumption, prothrombin time, and thromboplastin generation were performed by the standard methods described by Biggs & Macfarlane (1). Bleeding time was measured by the Ivy method (1). Normal bleeding time by this method, in our laboratory is between to 7 minutes. Plasma fibrinogen was determined by the method of Ratnoff & Menzie (24).

In vivo platelet adhesiveness was determined by the method of Borchgrevink (?). Clot retraction was estimated in hole blood and in PRP as described by Fonla (7). It was repeated after addition of. Mg++ 10^{-}[illegible] M ADP 10^{-}[illegible] M ADP 10^{-}[illegible] M+Mg 10^{-}[illegible] M and ATP 10^{-}[illegible]+Mg 10^{-}[illegible] M Platelet factor 3 availability vaccous metamorphosis and platelet aggregation tests were done according to Hardisty (13). Platelet aggregation to connective tissue was performed according to Zucker (30). Platelet factor 3 availability was also estimated by the method of Spaet & Cintron (27).

ADP-induced platelet adhesiveness was determined by modification of the procedure previously described (6). ADP was prepared as stock solution of 1 mg/ml in tris-NaCl buffer (1 vol. of tris HCl buffer 0.2 M pH 7.4+ 9 vol. NaCl 0.85%), deep frozen in samples of 0.5 ml, thawed and diluted with the same buffer on the day of the experiment. Four siliconized tubes were used. T 3 tubes containing 10 mg of washed glass powder (Fisher products) ere added 0.9 ml of PRP and 0.1 ml of ADP solution, the final concentrations being 1 µg/ml, 0_ µg/ml, respectively The fourth tube which was used as a control, contained 0.9 ml of PRP and 0.1 of tris-NaCl buffer The tubes ere shaken for 1 minute at room temperature and then 5 minutes were allowed for sedimentation of the glass powder A platelet count as taken from the center of the supernatant plasma and percentage platelets adhering to the glass for each concentration of ADP as calculated by comparing to the control tube. Results for different ADP concentrations in 15 normal subjects each, showed at ADP 1 µg 1 ml—platelet adhesiveness 89% ± 5 at ADP 0_ µg ml—75% ± 7 at ADP 0 1 g ml—57 ± 8.

Platelet fibrinogen as determined by the method of Ratnoff & Menzie (24) on the extract of digitonin-lysed platelets (30). A suspension of at least 10^9 platelets (in 0.1 ml buffer) was assayed The mean normal range according to the literature is (22) 150–180 µg fibrinogen per 10^9 platelets, in 3 normal subjects we found by this method 140 145 and 160 g 10^9 platelets.

CASE REPORTS

A 7 year-old boy (A N) was referred to the Pediatric Hematology Clinic with numerous diffuse petechiae and ecchymoses following trauma. At circumcision performed on the 8th day after birth he had bled profusely At the age of 10 months

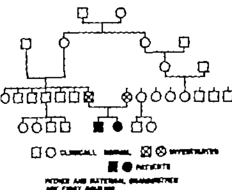


Fig. 1. A pedigree of the family N with thrombasthenia.

minor abrasions of the lips and tongue led to severe bleeding which lasted 4 days and necessitated blood transfusion. Since then he had recurrent epistaxis and bruising following trauma.

His younger sister (O. N.), now $4\,^1/_2$ years old, developed severe purpura immediately after birth. Ecchymoses after minor trauma have occurred since then. Recently she was involved in a car accident causing severe bleeding from lacerations of the upper lip and a huge hematoma of the face. The bleeding was controlled by transfusion of fresh plasma and platelet concentrate. In both children there was no history of hemarthrosis or visceral hemorrhage. The family history disclosed consanguinity: the maternal grandmother and the father are first cousins (Fig. 1). There was no family history of bleeding tendency.

RESULTS

Coagulation studies performed on the children (A. N. and O. N.) and parents (N. N. and S. N.) are summarized in Tables 1, 2 and 3.

The data in Table 1 show in both children a prolonged bleeding time, normal platelet count, absence of clot retraction (Fig.) impaired viscous metamorphosis and platelet aggregation after addition of thrombin and connective tissue. *In vivo* platelet adhesiveness was impaired, as well. Clotting time, prothrombin time, plasma fibrinogen, prothrombin consumption and thromboplastin generation were normal. Extractable platelet fibrinogen was absent in both children, however, as the fibrinogen method used is not as sensitive as the immunological method, we could not rule out traces of fibrinogen. Addition of

Table 2. *Platelet factor 3 availability according to Hardisty (31)*

Mixture		
PRP	PPP	Clotting time, sec
Control	Control	36
O. N	Control	49
Control	O. N	35
O. N	O. N	51
A. N	Control	55
Control	A. N.	37
A. N	A. N.	57
M. N	M. N	35
S. N	S. N	36

Table 1. *Hemostatic tests*

	O. N. (sister of A. N.)	A. N. (brother of O. N.)	M. N. (mother)	S. N. (father)
Clotting time in ordinary glass, min	4	6	4½	3
Bleeding time (Ivy), min	20	20	2	4
Platelets per cu. mm, × 10³	189	195	160	167
Prothrombin time, %	80	90	100	85
Recalcification time, sec	180	112	120	185
Prothrombin consumption index, %	10	11	10	60
Plasma fibrinogen, mg %	295	300	350	320
Platelet-extracted fibrinogen, μg/10 platelets	0	0	155	145
Platelet-TGT at the 4th minute, sec	10.4	9.8	9	10
In vivo platelet adhesiveness (normal 30–40 %)	14	22	30	35
Viscous metamorphosis	Impaired	Impaired	Normal	Normal
Platelet aggregation to connective tissue	Impaired	Impaired		
Clot retraction, %				
Whole blood	0	0	100	100
PRP	2	0	100	100
PRP + Mg (10⁻³ M)	36	18		
PRP + Mg (10⁻³ M) + ADP (10⁻³ M)	36	15		
PRP + Mg (10⁻³ M) + ATP (10⁻³ M)	36	15		

Table 3 *ADP-induced platelet adhesiveness*

Subject	Percent adhesiveness ADP 1 µg/ml	ADP 0.2 µg/ml
O N.	11	15
A. N	17	19
M N	80	71
S. N	89	74

magnesium with ATP and magnesium with ADP partially corrected the clot retraction more so in patient O N (Fig. 3). Platelet factor 3 availability tested according to Hardisty (15) (Table _) and to Spaet (Fig. 4) as well as ADP induced-platelet aggregation and adhesiveness were impaired in both children. All coagulation studies in the parents were normal.

DISCUSSION

Blood platelets have an important function in the first stages of hemostasis. They adhere to the subendothelial connective tissue fibers present on the surface of injured blood vessels (16) followed by subsequent aggregation.



Fig Clot retraction of recalcified platelet-rich plasma. N M Mother N S father N A son, N O daughter

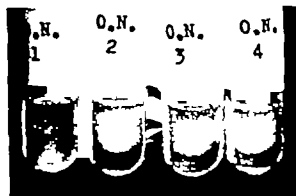


Fig 3 Clot retraction in recalcified platelet-rich plasma of patient N. O 1 PRP 2 addition of ATP Mg; 3 addition of Mg; 4 addition of ADP + Mg

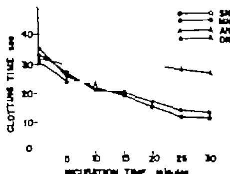


Fig 4 Platelet factor 3 availability according to Spaet & Cintron (14). Patient samples (AN and ON) show no activation by kaolin; the parents samples (SN and MN) show activation. Kaolin was added to PRP at time zero. Aliquots were mixed with Russell viper venom and calcium chloride at times indicated on abscissa.

Hellem (15) and Gaarder *et al* (8) established the role of adenosine diphosphate (ADP) in platelet aggregation. ADP derived from damaged endothelium or intrinsic ADP liberated from platelets by thromb n, induce this phenomenon (18) Platelet cations, Mg^{++} and Ca^{++} serve as cofactors. Adhesion to the damaged endothelium and aggregation of platelets result in the formation of a platelet plug. Thereafter the contraction of the platelet mass triggered by thrombin consolidates the plug. Released platelet factor 3 contributes to further thrombin formation and strengthening of the platelets mass.

Recently new methods for the study of platelet function have been developed. Among the most important ones are determination of platelet aggregation and adhesiveness induced by ADP (13 15 16 31) platelet factor 3 availability (13 27), platelet fibrinogen (5 17 _) and platelet enzymes (4 11 _1) Using these techniques, thrombasthenia has been defined as a distinct clinical entity (4 13) and was differentiated from other clinically similar congenital qualitative platelet disorders. Thrombasthenia is characterized by normal platelet count, prolonged bleeding time, absent or impaired clot retraction, a striking defect in ADP induced platelet aggregation and adhesiveness, low or absent platelet fibrinogen (5 _2) In some cases decreased platelet factor 3 availability (4 13) and platelet enzyme defects are found, namely reduced activity of ATPase, pyruvic kinase, glyceraldehyde phosphate dehydrogenase ATP concentration may be low (11 21). In other studies the level of nucleotides and of the above enzymes were found

to be normal (4 9 20) These investigations also excluded defective glycolysis and low concentration of ATP as a cause of the poor clot retraction of whole blood or platelet rich plasma.

The two cases described satisfied currently accepted diagnostic criteria. The hemorrhagic manifestations occurred early in life and bleeding was confined to the skin or mucous membranes. In contrast to hemophilia, visceral hemorrhage or hemarthrosis were absent. Study of the parents revealed normal blood coagulation and platelet function. The occurrence of the disease in brother and sister the negative findings in the parents and the consanguinity point to an autosomal recessive mode of inheritance in this family This is in accord with reports on other families reported (23). As in the presently examined family it is not yet possible with current techniques to detect the heterozygous state (4).

The hemostatic abnormality in the two patients is manifested by disturbed ADP—induced platelet adhesiveness, impaired in vivo platelet adhesiveness, platelet viscous metamorphosis, platelet aggregation to connective tissue, platelet factor 3-availability and low platelet fibrinogen. Evidence is available to indicate that the lack of platelet reactivity to intrinsic or extrinsic ADP results from a defect of the platelet surface membrane (4 19 13). However the exact site and mode of action of ADP on the platelet have not yet been determined (9 12, 25 28)

The significance of impaired platelet factor 3 availability in thrombasthenia is still debated. According to Hardisty (13) thrombasthenic platelets do not release factor 3. Caen and his co-workers (4) attribute this phenomenon to the lack of platelet aggregation which is responsible for their poor prothromboplastic activity Apparently the tests for thromboplastin generation and prothrombin consumption, which were normal in our cases, are generally insensitive to reveal a disturbance in factor 3 release. However in some reported patients with thrombasthenia, prothrombin consumption was found impaired (13 32)

Recent studies (4 5 17 22, 32) have shown platelet fibrinogen to be decreased in thrombasthenia. The fibrinogen deficiency has been thought to be responsible for the impaired platelet aggregation but proof of this is still lacking.

Whatever the platelet defects in thrombasthenia, they cannot as yet be corrected, and the only effective treatment thus far consists in the administration of fresh platelet rich plasma or platelet concentrates.

SUMMARY

Clinical and laboratory observations on a brother and sister with congenital hemorrhagic disorder are presented. Both had prolonged bleeding time, poor clot retraction and failure of the platelets to aggregate and adhere to glass in the presence of ADP absence of platelet extractable fibrinogen and impairment of platelet factor 3 availability The abnormal platelet function, confirms the recent diagnostic criteria of thrombasthenia Coagulation studies in the parents were normal. The hemostatic abnormality in thrombasthenia is discussed.

ACKNOWLEDGEMENT

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REGIONAL LUNG FUNCTION STUDIED WITH ^{133}Xe AND EXTERNAL DETECTORS (RADIOSPIROMETRY) IN CHILDREN WITHOUT CARDIOPULMONARY DISEASE

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Methods for analysing regional lung function, such as bronchospirometry and lober spirometry are difficult to perform in children. Methods using radioactive gases and external detectors might therefore be valuable in this age group. In fact, it has been claimed that one of the chief indications for the clinical use of ^{133}Xe is pulmonary disease in children (3).

The various pulmonary function tests using ^{133}Xe as a tracer substance in conjunction with external detectors are based on the fact that ^{133}Xe is poorly soluble in blood. When injected intravenously about 90 per cent will pass from the blood to the alveoli during its first circulation through the lungs, the amount reaching a given part of the lung being proportional to the blood flow to that part. When a subject inhales ^{133}Xe from a spirometer most of it will remain in the alveoli (again because of its poor solubility in blood) and the amount arriving at each part of the lungs with each breath is proportional to the ventilation of that part.

A method for measuring regional lung function with ^{133}Xe in adults has been developed at the department of Clinical Physiology at Malmö General Hospital (1 8 9). This technique, called radiospirometry has been tested against bronchospirometry in adults with various pulmonary diseases, and found to give the same or more detailed information about regional lung function (9). Since the radiation dose is small (7) it was thought legitimate to adopt and test the method in the examination of pulmonary function in children (6).

In this study children without cardiopulmonary disease were investigated to get information about normal distribution of ventilation and perfusion and to obtain a reference for findings in children with disturbed pulmonary function.

METHOD

Since the details of the equipment and the collimation have been described by Mörner (9) only short description will be given here.

The collimation developed for adults was also used for the children. The apex and the diaphragm ere localized by fluoroscopy image amplifier and TV and their projections were marked on the skin. The child was placed in the midplane between 4 anterior and 4 posterior detectors as appears from Figs 1 and 2. There was thus some overlapping between the apical and the basal field but little overlapping between the right and left side. The detectors were coupled in pairs (Fig. 1).

Before the examination the children ere accustomed to the investigator and trained with the aid of spirometer for those phases of the examination requiring cooperation, for instance apnea after normal inspiration.

As the results depend very much on fixed relationship bet een the subject and the detectors throughout the examination, the position of the subject was carefully controlled during the whole procedure by the aid of spotlights sharply focused on ink dots on the skin.

About half an hour before the onset of the examination a short teflon catheter[1] (4) was inserted percutaneously into the femoral vein and physiological saline was repeatedly injected to convince the child that the injections were painless. The mouthpiece of the spirometer system (see below) was placed in the child' mouth but not connected to the system.

About 0.5 ml of saline solution containing 0.1–0.2

[1] A teflon catheter with three-way stopcock, length 7 cm, external diameter 1.45 mm. Available from Stille-Werner Stockholm, Sweden.

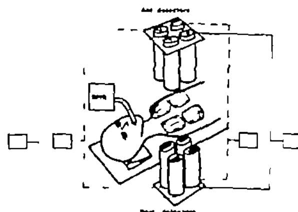


Fig 1 Sketch, showing the arrangement of the 4 anterior and the 4 opposing posterior detectors. The detectors are coupled in pairs The axillary plane of the subject is in the horizontal midplane between the two sets of detectors. The vertical distance bet een the collimators and the axillary line was 17 cm. *SPIR*, Closed circuit spirometer *R*, recorder

mC ^{133}Xe[a] was rapidly injected into the cathete immediately followed by 5–10 ml isotonic saline. After the injection the subject was instructed to hold his breath for about 10 seconds in end tidal inspiratory position. Before the investigation the children were taught how to hold

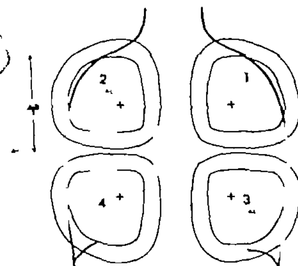


Fig 2. Sketch, showing the fields covered by the detectors in the horizontal midplane between the 4 anterior and the 4 posterior detectors. The contours of child with the mean measurements of the chest (Table) are drawn. The 90% and the 50% isocount lines of each field are marked, + Indicates the projections of the optical centers of two opposing detectors; *A-D* the distance of apex-diaphragm (maximal inspiratory position)· *A D*/2, half the distance of apex-diaphragm. The figures in the fields are used in the calculations to characterize the fields.

then breathe in end tidal inspiratory position, this position being much easier for them, especially the younger ones, to maintain than the resting expiratory level, i.e. that used for adults (9). To establish the reproducibility of the measurements of regional perfusion, second injection was given after counting rate had returned to back ground level. Each child thus received two injections. For technical reasons in two of the children the injections were given into a brachial vein.

When the counting rate returned to background level after the second injection the child was coupled to closed circuit spirometer containing ^{133}Xe (about 0.2 mC/l)[a] and a carbon dioxide absorbent to which system oxygen was added in order to keep the olume constant. The child rebreathed then in this system until the counting rate had become stable in all the channels, after which he performed a maximal inspiration and a maximal expiration.

The disappearance of ^{133}Xe from the lungs after the injections and after rebreathing procedure was found to be rapid. The exposure to radioactivity was thus short and it was thought legitimate to make double determinations of the first three breaths in the spirometer system. Such duplicate determinations were performed in 7 children.

CALCULATIONS

The calculations are made under the assumptions that the total olume of the lungs is covered by the detectors and that the total ventilation and perfusion are registered ith fairly uniform sensitivity in the volume seen by each pair of detectors These assumptions are based on investigations in adult where radiospirometry was compared ith bronchospirometry (9).

The radioacti ity in the lungs was recorded in four channels, one for each of the four lung fields. The same amplification and discrimination was used for the 4 channels. Reproductions of the records from the 4 lung fields are shown in Fig. 3 I the records the various distances (Q, V2, etc) are used as primary measurements of the activity registered in the fields. In the records, reproduced in Fig. 3, the following distances were thus measured for the right pical lung field.

Q – The distance from background counting rate to the level of counting rate recorded during apnea in FRC + V position after the injection of ^{133}Xe. This distance was taken as measure of the perfusion of the lung field.

V2 – The distance from background counting rate to the level reached afte the first 3 normal breaths in the closed circuit spirometer system. This distance as taken as measure of the entilation of the lung field.

From the Radiochemical Centre, Amersham, England, or AB Atomenergi, Studsvik, Sweden. The doses given refer to the Swedish ^{133}Xe solution hich gives 3–5 times higher counting rates than the English.

50% of the activity disappeared ithin 13.4 seconds ± 4.6 seconds (M ± S.D.) during the wash out after the first injection.

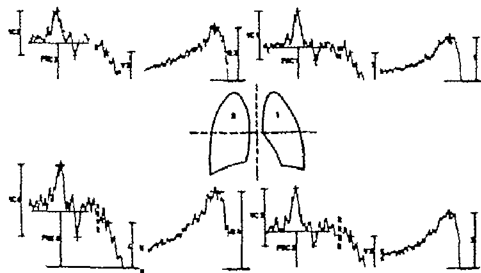


Fig. 3 Reproduction of the recordings obtained from the 4 channels. The designations of the distance are explained in the text

FRC 2 = The distance from background counting rate to the level reached after attained stable level of counting rate during rebreathing in the spirometer system. This distance was taken as measure of the lung volume (functional residual capacity) of the lung field.

VC 2 = The distance from the counting rate at maximal expiration to the level of counting rate at maximal inspiration. This distance was taken as measure of the "vital capacity" of the lung field.

The designations given in Fig. 3 are used for the other lung fields.

With these distances it is possible to calculate the percental relation of the function of one lung field to the sum of the function of that lung field and its corresponding lung field on the opposite side: e.g. perfusion of right apical field in relation to the perfusion of both apical fields

$$Q\,Ra = \frac{Q2}{Q1+Q}\ 100 \qquad (1)$$

ventilation of right apical field in relation to the ventilation of both apical fields

$$V\,Ra\ \frac{V2}{V1+V2}\ 100 \qquad (2)$$

FRC and VC of lung field were calculated in the same way. The designations for the right apical lung field are FRC *Ra* and VC *Ra*, respectively.

Corresponding designations for the right basal lung field are Q *Rb*, V *Rb*, FRC *Rb*, VC *Rb*.

For the right lung perfusion, ventilation and lung volumes are calculated according to the formulas

$$Q\,R\ \frac{Q2\ \ Q4}{Q1\ \ Q\ \ +Q3+Q4}\ 100 \qquad (3)$$

$$V\,R = \frac{V2+V4}{V1\ \ V2\ \ V3+V4}\ 100,\ \text{etc.} \qquad (4)$$

Only relative values are thus obtained. This means that if one lung has decreased function of the same degree and localization as the opposite lung the results of radiospirometry may be normal.

With the distances given in Fig. 3 it is also possible to calculate the relation of the function of one lung field to that of all the 4 fields. For the right apical lung field the formulas are:

$$Q\,II\ \frac{Q2}{Q1\ \ Q2\ \ Q3+Q4}\ 100, \qquad (5)$$

$$V\,II = \frac{V2}{V1+V2\ \ V3-V4}\ 100 \qquad (6)$$

$$FRC\,II\ \frac{FRC2}{FRC1-FRC2+FRC3-FRC4}\ 100 \qquad (7)$$

These formulas can be used for the calculation of ventilation and perfusion indices (and) for each lungfield or each lung.

For the right apical lungfield the formulas are

$$\text{perfusion index and } Q\,II = \frac{Q\,II}{FRC\,II} \qquad (8)$$

$$\text{ventilation index and } V\,II\ \frac{V\,II}{FRC\,II} \qquad (9)$$

A quotient of ventilation to perfusion can also be calculated for each lung field and each lung. For the right apical lung field this quotient is

$$V\,Q\,II\ \frac{V\,II}{Q\,II} \qquad (10)$$

For the calculations of these indices and quotient only the first measurements of perfusion and ventilation are used.

MATERIAL

The material consisted of 1. children—6 girls and 6 boys. They were selected f

Table 1 *Clinical data of 12 children used for ascertaining normal values of radiospirometry*

Subject No.	Sex	Age	Clinical diagnosis	Chief symptoms	Chest investigations performed	Comments	Height (cm)	Weight (kg)	Measurements of thorax, cm Width	Sagittal	Apex depth
1	M	7 /	Exogenous intoxication	Drowsiness	ECG X-ray lungs. Spirometry	Investigated when healed	121	[illegible]3	20	13	14.5
2	M	11 $/_{12}$	Constitutional dwarfism	Shortness of stature	ECG X-ray lungs. Spirometry	Normal body proportions	127	6	21	14	15.5
3	M	13 $/_{12}$	Cardiac neurosis	Chest pain	ECG X-ray heart & lungs. Spirometry	No somatic abnormalities	151	47	25	17	20.0
4	M	13 $^{8}/$	Paroxysmal tachycardia?	Chest pain	ECG X-ray heart & lungs. Spirometry	No somatic abnormalities	155	36	23	17	22.0
5	M	13 $/_{12}$	Terminal ileitis	Diarrhoea	ECG X-ray lungs. Spirometry	In good general condition	157	39	23	18	19.0
6	M	14 $/_{12}$	Celiac disease	Shortness of stature	ECG X-ray lungs. Spirometry	Routine check-up	147	33	21	16	19.0
7	F	9 /	Psychasthenia	Emotional instability	ECG X-ray heart & lungs. Spirometry	Submission to child. psychiatr	130	25	19	13	16.0
8	F	10 $/_{12}$	Acute osteomyelitis	Leg pain	ECG X-ray lungs. Spirometry	Investigated when healed	137	27	20	14	16.0
9	F	10 $/_{12}$	Cardiac neurosis	Chest pain	ECG, X-ray heart & lungs. Spirometry	No somatic abnormalities	150	35	22	15	17.0
[illegible]	F	10 $/_{12}$	Abdominal pain (psychogenic)	Abdominal pain	ECG, X-ray heart & lungs. Spirometry	No somatic abnormalities	149	33	2[illegible]	15	17.0
11	F	11 $/_{12}$	Acute pyelitis	Painful micturation	X-ray lungs. Spirometry	Investigated when healed	146	31	20	15	15.0
12	F	14 $/_{12}$	Cardiac neurosis	Chest pain	ECG X-ray heart & lungs. Spirometry	No somatic abnormalities	161	40	23	16	18.5

treatment or investigation at the Department of Pediatrics, University Hospital of Lund (Table 1). None of the 12 children had heart or lung disease. In 6 children (nos. 3 4 7 9 10 and 12) chest disease had at first been suspected but examinations for organic chest disease had revealed nothing remarkable. Furthermore, 5 of these 6 children showed evidence of psychosomatic distress. Follow up $^1/_2$–1 $^1/_2$ years did not give any reasons to suspect organic disease. Two of the 12 children (nos. 8 and 11) had an acute infectious disease (osteomyelitis of the lower limb and a urinary tract infection, respectively). They were examined with radiospirometry just before dismission when their infections had healed.

One child (no. 6) had a celiac disease and had been treated with gluten-free diet since about one year. At the time of the investigation he was symptomfree and in hospital for a routine check up.

One child (no. 5) had regional ileitis for two months. At the time of radiospirometry he had

Table 2. *Measurements of the chest in 12 children, investigated with radiospirometry*

	Mean	Range	S.D.
Width	21 6	19 .3	1 73
Sagittal	15.3	13 –18	1 60
Apex-diaphragm	17.5	14.5–.2.0	2.26

no symptoms. The blood values were normal but ESR was moderately increased.

The size of the chest was determined with 3 measurements:

Sagittal. The sagittal diameter at mamillary level.

Width. The width at mamillary level.

The distance between lung apex and diaphragm. Measured with the aid of fluoroscopy with the child recumbent and at maximum inspiration. These measurements are given in Tables 1 and 2 for comparison with corresponding measurements in children with pulmonary disease, intended to be published later on.

RESULTS

Table 3 gives the values for regional perfusion, ventilation and functional residual capacity of the right lung and its two fields. Corresponding values for the left lung are *100 minus the values found* for the right lung.

Table 4 gives the indices of ventilation (Ind V) and perfusion (Ind Q) and the quotients of ventilation to perfusion (V/Q).

DISCUSSION

The mean values of Q V VC and FRC found for the right lung are higher than those for the left lung, a finding in accordance with the results of bronchospirometric studies in healthy adults (5 10). The mean values in the present study agree well with corresponding means obtained with bronchospirometry in adults (10).

Comparison of the present results with those obtained with the same technique in healthy adults (9) showed agreement of the means and the S.D.-values of the parameters reported in Table 3. Tests of significance[1] revealed no difference between the results in the children and those in the adults ($p > 0.1$).

In both studies ventilation and perfusion per unit lung volume (functional residual capacity) = the indices of ventilation and perfusion—for the right lung were lower than those for the left (present study $p < 0.001$). A similar tendency was seen at bronchospirometry in adults (10). In the *present* study (as in the previous study in adults (9)) the indices of ventilation and perfusion for the right basal field were lower than those for the left basal field ($0.05 > p > 0.01$). The dif

Table 3. *Perfusion, ventilation an... nctional residual capacity of the apical field, the ...al field and the right lung in 12 children*

The results are given as percentages of ... r of the right field and corresponding field of the op... side. The figures within brackets denote the ... tained in 38 healthy adults, investigated by Moore

Parameter right lung	Mean	Range		ε
Perfusion (Q)				
Single determ.				
Q Ra	52.4 (52.4)	48.9–56.9	2 4 3 2	—
Q Rb	52.3 (52.9)	49.4–54 7	2 5 2	—
Q R	53.8 (52 6)	49 7 56 3	2 2 2	—
Duplicate determ. (n = 12)				
Q Ra	52.5		49.8–56.9	2 2	1 3 1 3
Q Rb	53 3	48 1 56 5	2.2	1 4 (1 0
Q R	53 1	49 6–56.0	1 9	0 8 0 8
Ventilation (V)				
Single determ.				
V Ra	51 4 (50.5)	49 6–55 1	1 6 (2 8	—
V Rb	54.2 (54 .)	49 8–59 4	8 (3 6)	—
V R	53 3 (52.5)	50 5–56 5	0 (2.6	—
Duplicate determ. (= 7)				
V Ra	52.2	50.1 54	1 4	2 7 (1 7)
V Rb	54.4	51 8–57 6	2 3	1 6 (2 0
V R	53 7	52 0–56 1	1 3	1 4 (1 3
Vital capacity (VC)				
Single determ.				
VC Ra	51 6 (49 9)	45 7–59 2	3 7 (2 7)	—
VC Rb	54 0 (53 7)	50.5–57 7	2 6 (3 0	—
VC R	53 0 (52.0)	49 0–57 6	2 5 (2 1)	—
Functional residual capacity (FRC)				
FRC Ra	52.8 (52.9)	49.5–56.4	2 5 (2 8)	—
FRC Rb	55.8 (56.4)	52.2 58 0	1 7 (3 1)	—
FRC R	54 9 (54 8)	52.5–56 3	1 3 (2.5)	—

Error of single determina ion $\sqrt{\sum d^2 / 2n}$.

[1] Student' test.

Table 4 *Indices of ventilation and perfusion and the quotients of ventilation to perfusion in 12 children*

Within brackets the values obtained in 38 healthy adults (9). Indicates those results, where probably significant ($0.05 > P > 0.01$) difference was obtained between the children and the adults, and those results where a significant ($0.01 > P > 0.001$) difference was found

	Right lung			Left lung		
	Mean	Range	S.D.	Mean	Range	S.D.
Index of ventilation (Ind V)						
Apical	1.01 (0.95)	0.86–1.21	0.13 (0.08)	1.06 (1.05)	0.95–1.24	0.08 (0.10)
Basal	0.96 (0.97)	0.90–1.06	0.05 (0.06)	1.03 (1.06)	0.83–1.17	0.08 (0.11)
Total	0.97 (0.96)	0.91–1.04	0.03 (0.04)	1.04 (1.05)	0.95–1.11	0.04 (0.06)
Index of perfusion (Ind Q)						
Apical	1.07 (0.97)*	0.90–1.41	0.14 (0.08)	1.08 (1.00)	0.93–1.28	0.11 (0.11)
Basal	0.92 (0.95)	0.80–1.03	0.08 (0.06)	1.02 (1.10)*	0.89–1.16	0.08 (0.10)
Total	0.97 (0.96)	0.91–1.04	0.04 (0.04)	1.04 (1.05)	0.96–1.11	0.04 (0.05)
Ratio of ventilation to perfusion (V/Q)						
Apical	0.96 (0.98)	0.74–1.14	0.14 (0.08)	1.00 (1.05)	0.82–1.14	0.09 (0.12)
Basal	1.05 (1.02)	0.95–1.21	0.09 (0.07)	1.02 (0.97)	0.78–1.24	0.13 (0.10)
Total	1.01 (1.00)	0.92–1.11	0.06 (0.04)	1.00 (1.00)	0.89–1.10	0.07 (0.05)

ferences between the indices for the apical fields were not statistically significant ($p > 0.1$).

The calculation of the indices assumes that the distribution of lung volume among the 4 fields is the same in end tidal inspiratory position (FRC + V_T position) as in resting expiratory position (FRC position). This assumption is corroborated by calculations where FRC *Ra* and FRC *Rb* were ... d with measurements using the distances ... background counting rate to the level of counting rate obtained in FRC + V_T position during rebreathing to "steady state". The mean ± S.D. of the differences [FRC *Ra* − (FRC + V_T)*Ra*, etc.] were only +0.8% ± 1.6% (right apical field) and −0.7% ± 1.1% (right basal field).

More important, however, is that in using these indices one neglects the influence of the activity from ^{133}Xe absorbed by the blood in the lungs during the rebreathing to "steady state" and transported to different tissues in the body. If the tissue ^{133}Xe is not distributed in the same way as the ^{133}Xe in the alveoli, it will influence on the calculations of lung volume. Due to the position of the liver it is possible that the activity of tissue ^{133}Xe recorded by the detectors of the right basal field is higher than that recorded by the detectors of the left basal field, the detectors of these fields cover also the upper part of the abdomen, especially in the younger children. If the quotient between the tissue ^{133}Xe of the right basal field and the left basal field exceeds the quotient between the "true" FRC of the right basal field and the "true" FRC of the left basal field the calculated *FRC* of the right basal field will be *overestimated*. Consequently the calculated *indices* of that field will be *underestimated*. The lower indices found for the right total lung and the right basal field might thus partly be due to the influence of tissue ^{133}Xe.

The indices of perfusion for the right apical field and for the left apical and basal fields differed (for right apical field, $0.01 > p > 0.001$, for the other $0.05 > p > 0.01$) from corresponding indices of adults (Table 4), which might be due to different influence of tissue ^{133}Xe in the groups of subjects.

As information of pulmonary function during *normal* breathing was desired the measurements of the V and Q parameters were performed in FRC + V_T position. This position is more difficult to define than the TLC and the FRC positions. However, the TLC position was not used since, especially in patients with lung disease, the distribution of ventilation and perfusion in that position may differ considerably from the distribution during normal breathing (2). Furthermore, the tendency to make a Valsalva maneuver is greater in that position. The FRC position was omitted since the children had difficulties in maintaining apnoea long enough for the injection of

^{133}Xe solution in that position. Though only ocular control of the FRC + V_T position was performed after the injection of ^{133}Xe, this position was thus used.

The errors of a single determination[1] of the perfusion and the ventilation parameters (*Table 3*) are of about the same magnitude as those found in healthy adults (9). They include the errors of the apparatus, changes in position of the subject during the procedure and true changes in ventilation and perfusion within the lungs. With regard to these factors the errors found must be regarded as small and the reproducibility of the results on one and the same occasion of examination as high. For duplicate examinations at several days' interval Miörner found in adults a reproducibility almost as high as for examinations on one and the same occasion (9).

SUMMARY

Regional lung function was studied in 12 children without cardiopulmonary disease. ^{133}Xe was used as a tracer and external detectors were employed. Perfusion, ventilation and functional residual capacity of an apical field and a basal field of each lung were measured. The results agreed closely with those obtained in healthy adults, studied with the same technique.

The reproducibility of the results on one and the same occasion was high as judged from duplicate determinations.

Regional lung function in relation to regional functional residual capacity (indices of ventilation and perfusion) and the quotients of ventilation to perfusion of the lung fields were also calculated and were compared with the same measurements in adults. Concerning the perfusion indices for the different lung fields certain differences were found between the two groups of subjects.

As the normal range of the different radiospirometric measurements was small the method seems suitable for detecting regional abnormalities in children with lung disease.

[1] According to the formula, $= \sqrt{\Sigma d^2/2n}$, where d is the difference between paired determinations and n the number of differences.

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LOW BIRTH WEIGHT THE "SMALL FOR DATES" SYNDROME AND PERINATAL MORTALITY IN A LOW FAMILY INCOME GROUP

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The influence of low family income and lack of ante-natal care on birth weight and neonatal complications in the poor urban population of Pakistan is unknown. This survey was made in order to get closer to an evaluation of the importance of this particular problem.

MATERIAL AND METHODS

1100 consecutive deliveries from 1st of August 1964 to 31st of May 1965 were studied. The mothers in the low income group were exclusively from a refugee area in Karachi. The fewer number of mothers from the upper income groups was taken from the Karachi middle-class of government employees. Ante-natal care was mostly negligible, had attended at irregular intervals. The iod of gestation was sometimes difficult to assess with in the lower income group of women, ho were mainly illiterates. In 928 out of the 1100 cases the family income was less than Rs. 300 or U.S. $ 60 a month. The incidence of consanguinous marriages was high, 42% were first cousin marriages.

All newborns were weighed by resident or a post graduate student within 4 hours of birth, and detailed examination of the newborn was made. Maternal history was then taken and the obstetrical history checked in conference with the mother

RESULTS

Birth weights are shown in Table 1 Prematurity or birth weight below 5 lb 8 oz (2500 g) was present in 22% of the cases Average birth weight was 6.2 lb 7% were below 4 5 lb or premature as suggested by Indian workers (5) Birth weight in relation to gestational age is given in Table 2 Birth weight 5 lb 8 oz (2500 g) or below was present in 117 out of the 694 newborns over 36 weeks of gestational age. Thus the "small for dates" incidence under these criteria was 17% Table 3 shows birth weights in relation to income groups. Prematurity" rate was 17% in the higher income groups (over 300 Rupees or 60 U.S. dollars a month)

Gross congenital abnormalities were found in 19 cases, out of which 1[illegible] were "premature by weight. Consanguinous marriages were present in 9 out of the 19 cases. 9 of the mothers were primiparae.

Still birth rate was 4 4% (see Table 4). 45 of the 48 cases were in the lower income group. No autopsies were allowed. In half of the still birth cases the period of gestation was given to be over 36 weeks. Chief causes of still birth were ante-partem haemorrhage mal-position of infant or dis-proportion and pregnancy toxaemia.

Neonatal mortality (born alive after 8 weeks of gestation and dying during the first week of life) was 5% Among the 103 cases (9 4%) of intrauterine and neonatal deaths, 63 cases were "premature by weight. In 16 of the 55 neonatal deaths no other cause than prematurity" could be found. Birth trauma and asphyxia were present in 19 cases. Gross congenital defect, RDS, aspirations and infection could be diagnosed in 5 cases each No autopsies were allowed.

DISCUSSION

The data were collected in a government—run obstetrical unit with a possible overrepresentation of patients belonging to low income groups. Due to the prevailing preference for home deliveries there could be an overrepresentation of complicated pregnancies and deliveries in this series. Ante-partum haemorrhage is thus for example a common cause for hospital delivery which fact should increase the "prematurity" rate found. It is realized that the true figures can only be obtained by a combined hospital and field study

Table 1 *Births weights and sex ratio*

Weight				No of cases	%	Sex ratio	
Lb	Oz	Lb	Oz			Male	Female
3	8			27	2.45	8	19
3	9	4	8	51	4.63	20	31
4	9	5	8	167	15.18	77	90
5	9	6	8	346	31.44	183	163
6	9 ~	7	8	362	32.90	222	140
Over 7	8			142	13.39	85	62
Total				1100	100.00	595	505

Prematurity rate 22.26 %

The incidence of low birth weight or pre maturity is considerably higher than that recently reported from an economically more developed country (3 7) and exceeds the one reported from Colombia (4). Average weight is 1 lb less than in babies delivered in the UK or USA. Even if the 2000 g level is used as a local criteria of "pre maturity due to hypothetial racial characteristics, the incidence of 7% is still higher than in the Scandinavian countries (5%).

The "prematurity"—incidence of 22% and the fact of 17% being of low weight, in spite of gestational age over 36 weeks, indicates that retarded intrauterine growth and not only abnormally short pregnancy might account for a consider able part of the low mean birth weight found. Period of gestation of less than 37 weeks has been found in only 12% among a low income group pregnancies in Lahore, Pakistan (1). A 24% prematurity" rate, according to weight, against 8% of less than 37% weeks gestational period was found in a recent study in India (2)

The drop from 2.% to 17% of premature" births between the lower and higher income groups is not considered significant due to the low number of patients in the upper income groups.

Table 2. *Weight in relation to period of gestation*

Gestation period	No of cases	Less than 3 lb 8 oz	3 lb 9 oz 4 lb 8 oz	4 lb 9 oz 5 lb 8 oz	Over 5 lb 8 oz
24 to 28 weeks	10	8	2	0	0
29 to 32 weeks	25	2	14	9	0
33 to 36 weeks	371	10	26	57	278
Over 36	694	7	9	101	577
Total	1100	27	51	167	855

Table 3 *Income-groups in relatio. birth weights*

Income	No of cases	Less than 4 lb 8 oz		Over 5 lb 8 oz
Rs. 0–100	320	33	6	22.
Rs. 100–300	608	31		401
Rs. 300–500	63	6	6	41
Rs. 500–700	97	—	7	90
Over Rs. 700	12	—	—	1
Total	1100	70	164	866

The domination of still births from the low in come groups is considered to reflect the lack of ante-natal care in these pregnancies.

The congenital abnormalities have been reported from a greater number of cases in an earlier paper (6).

The intrauterine and neonatal mortality is to be considered low in view of the lack of antenatal care and staff for proper new-born care. Most cases were sent home on the 3rd postpartum day and even if parents are known to consult the hospital staff in case of death or disease during the rest of the newborn period, the figure given must be considered as a gross minimum one

Table 4 *Neo-natal mortality in relation to birth-weight*

Birth-weight				Still birth	Neo-natal mortality	Peri-natal mortality
Lb	Oz	Lb	Oz			
Less than 3	8			3	18	21
3	9	4	8	12	8	20
4	9	5	8	10	12	22
5	9	6	8	13	10	23
6	9	7	8	9	5	14
Over 7	8			1	2	4
Total				48	55	103

In view of the known late neurological sequelae among prematurely born infants, the high "prematurity" rate in combination with a comparatively low perinatal mortality seems to give a high motivation for increased efforts in the direction of proper ante-natal care

SUMMARY

In 1100 consecutive deliveries the "prematurity" rate was found to be 22% Mean birth weight was 6.2 lb, or 2800 g. 17% showed a birth weight of less than 2500 g in spite of gestational age over 36 weeks Intrauterine mortality was 4% and neonatal mortality 5% Still births were predominantly in the antenatally neglected lower income groups and the causes of neonatal mortality is considered as mainly secondary to poor ante and post-natal care.

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LYSOSOMAL ENZYMES IN JUVENILE AMAUROTIC IDIOCY

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The site of the metabolic block in juvenile amaurotic idiocy (JAI) is unknown. By name as well as by symptoms and signs JAI resembles a group of other familial amaurotic idiocies (2). By the distribution of the histo-pathological changes JAI also has similarities to a new disease resembling Hurler's syndrome (5). This new disease as well as at least one or two of the entities in the group of amaurotic familial idiocies, e.g. infantile amaurotic idiocy (Tay Sachs disease) are storage diseases. In a series of storage diseases, including infantile amaurotic idiocy and the new disease mentioned, a defect of a catabolic enzyme, localized intracellularly to the lysosomes has been suggested (1 3–5).

There exists thus some evidence that a few diseases, in one way or the other with similarities to JAI, may be inborn lysosomal diseases (3)

Table 1 *Enzyme activities in post-mortem tissues*

All values expressed as μmoles substrate split/g wet weight/min

	β-Galactosidase (EC 3.2.1.23)	β-Glucuronidase (EC 3.2.1.31)	β-Acetylglucosaminidase (EC 3.2.1 30)	Acid phosphatase (EC 3.1.3.2)	-Mannosidase (EC 3.2.1 24)	-Fucosidase
Brain cortex						
Controls (n = 11–15) Mean ± s.d.	0 0676 ±0 0166	0.00591 ±0 00253	0.366 ±0 085	0.739 ±0 119	0.0409 ±0 0167	0 0312 ±0 0116
JAI						
Case 1 male, 20	0 0558*	0.00496	0.459*	1.25	0.0642*	0.0583
Case 2, male, 18	0 0627*	0.0292	0.714	1.56	0 0412*	0 0149*
Mean	0.0593	0.01708	0.587*	1 41 **	0 0527*	0.0366
Liver						
Controls (n = 13–14) Mean ± s.d.	0.646 ±0.225	0.396 +0.158	2.08 ±0 78	3.09 ±1.11	0 244 ±0 125	0.294 ±0 076
JAI						
Case 1 male, 20	1 446	0 880	2.44*	3.21	0 203*	0.356*
Case 2, male, 18	0 831	1.09*	2.72*	3.61	0.329*	0 132( )
Mean	1 139*	0.985	2.58*	3.41	0.266*	0.244
Spleen						
Controls (n = 12 14) Mean ± s	0.285 ±0 078	0.220 ±0.062	1 77 ±0.66	1.99 ±0 52	0 163 ±0.086	0.176 ±0 098
JAI						
Case 1 male, 20	0.189*	0.278*	2.40*	4 27	0.286	0 261
Case 2, male, 18	0 209*	0 470	3.76	1 74	0.106	0.0381
Mean	0.199*	0.374	3.18	3.02( )	0.196*	0.150*

(*)– Values differing significantly from the control values ( ) $p < 0.1$, $p < 0.05$, $p < 0.01$ $p < 0.001$
Not significantly different from the control values.

Table _ *Enzyme activities in liver biopsy specimens*

All values expressed as moles substrate split/g wet weight/min

	β-Galactosidase	β-Glucuronidase	β-Acetylglucosaminidase
Controls (= 12 15)			
Mean ± s.d.	0.649	0.523	1.83
	± 0 280	± 0.246	± 1 1
JAI			
Case 3, male, 17	0.923*	1 160	1 9
Case 4 female, 16	0 902	0.840*	1 76
Mean	0.913	1 000	1.84

This was considered motive for a study in JAI of the activity of several acid hydrolases, definitely or probably localized to the lysosomes.

MATERIAL AND METHODS

Plasma was sampled and analyzed as earlier described (6). Post-mortem tissues from tw cases of JAI had been stored at −20 for one (case 2) and up to tw (case 1) years and were kindly supplied by Dr A. Brun and Dr L. Svennerholm.

Fresh liver specimens were obtained by needle biopsy in local anaesthesia in tw patients with JAI. The tissue was immediately frozen and the analyses were performed within a few hours.

Control tissues ere obtained from adult patients at tion for cholelithiasis (liver) and five to fifteen post mortem (liver spleen, brain cortex) from adults and children deceased by accident or diseases considered not to engage primarily liver spleen or brain cortex. Sampling, storage and analyses for the control tissues were as in the JAI patients and as described earlier (6). Acid phosphatase was measured as will be described separately (7).

The patients with JAI all had been studied in detail for several years and the diagnosis was in ll cases considered to be definite.

RESULTS

Post mortem tissues Brain cortex, liver and spleen were analysed in two cases of JAI. The mean activity in these two cases of α-fucosidase and α-mannosidase was the same as in the controls in all three tissues, whereas that of β-galactosidase was high in liver but not in brain or spleen (Table 1). Acid phosphatase and β-acetylglucosaminidase were more active in brain and spleen but not in liver than in these tissues from the controls. β-glucuronidase was increased in all three tissues. No decreased mean activity for any of the enzymes measured was noted.

Liver biopsy specimens When liver biopsy specimens were analysed in two patients with JAI, the β-galactosidase activity was in the high normal range, not statistically significantly increased (Table ?) The activity of β-glucuronidase was significantly higher than and that of β-acetylglucosaminidase not different from the control values. Thus, a similar pattern, although somewhat less markedly pathological was seen as in the post mortem liver tissue from patients with JAI

Plasma. In plasma increased mean activities were found in the JAI patients for β-glucuronidase and also for β-galactosidase and, less marked, for α-fucosidase (Table 3) α-mannosidase was slightly less active than in the controls, while acid phosphatase and β-acetylglucosaminidase had a similar activity as in the controls.

Table 3 *Enzyme activities in plasma*

All values except for acid phosphatase expressed as μmoles substrate split/1000 ml min Acid phosphatase expressed as King-Armstrong units Statistical significances as in T ble 1

	β-Galactosidase	β-Glucuronidase	β-Acetylglucosaminidase	Acid phosphatase	α-Mannosidase	Fucosidase
Controls (n = 14–16)	0.240[a]	0.0689[a]	5.06[a]	2.54[a]	2 13[a]	3.62[a]
Mean ± s.d.	± 0.068	± 0.0300	± 1.28	± 0.73	± 0 58	± 1.82
JAI						
Case 3, male, 17	0 399	0.218	6 41	4.8	1 36	7 44
Case 4, female, 16	0.233	0.180	6.52*	1.5*	1 86	6.29*
Case 5 male, 20	0.499	0.383	5.44*	1.3	1 86	6.29*
Case 6, male, 19	0.316*	—	5.20*	—	1 14	—
Cases 3–6, mean	0 362	0 260	5.89*	_53	1 56	6.67

From Öckerman (6).

DISCUSSION

The finding of a slightly increased enzyme ac tivity must always be judged with extreme caution. Only when satisfactory sampling, storage and preparation for analysis, a correct method of analysis and suitable controls have been used, moderate deviations may be presumed to be real In this study it is probable that the finding of increased activities of a few enzymes in plasma and liver biopsy specimens mirrors the true *in vivo* situation in the JAI patients, although not necessarily directly due to the disease itself. The results on post-mortem tissues are less certain, since it is not possible to appreciate, what changes may have occurred post-mortem and during storage. However the finding of increased enzyme ac tivities would be more obviously non-artifactual than that of decreased activities.

The similarity of the results to those found in gargoylism (6) and a new disease resembling Hurler's syndrome (5) both these diseases supposedly being due to primary lysosomal enzyme defects, might motivate an intensified study of the role of the lysosomes in the pathogenesis of JAI

SUMMARY

The activity of several acid hydrolases was measured in tissues and plasma from patients with juvenile amaurotic idiocy (JAI) Increased activities were found for β-galactosidase, β-glucuronidase, β-acetylglucosaminidase, acid phosphatase and α-fucosidase. No significant changes were found for α-mannosidase, except slight decrease in plasma. The results show similarities to the findings in gargoylism and a new disease, resembling Hurler's syndrome. These two diseases are supposed to be caused by pr mary lysosomal enzyme defects. It is suggested th h role of the lysosomes in the pathogenesis of J I should be studied in more detail

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REVIEW ARTICLE

NEONATAL HYPOGLYCEMIA

II A Clinical Study of 44 Idiopathic Cases with Special Reference to Corticosteroid Treatment

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In 1959 Cornblath *et al.* drew attention to the syndrome of symptomatic neonatal hypoglycemia and pointed out its association with permanent neurological complications appearing later in infancy (8). Since then, this association has been confirmed by several workers (5 7 13), and the prevention (.3 27), early detection (6 7) and treatment (5 6, 10) of the disorder have been discussed in numerous reports.

The depression of blood glucose levels in the newborn can be caused by several etiological factors (for review see ref 9) In infants of diabetic mothers the phenomenon is probably sociated with hyperinsulinism (17), as also in the very rare cases of islet-cell adenoma in the neonate (e g. 18, —) In the terminal stages of fatal neonatal disorders hypoglycemia is not uncommon (6, 7 12). Certain inborn errors of metabolism may be associated with hypoglycemia already in the first days of life (9) and by an unknown mechanism, severe erythroblastosis can also be causative (14 19).

In the majority of cases, however no specific etiological factors can be demonstrated, and the disorder is classified as idiopathic. The purpose of this paper is to present the clinical characteristics and immediate results of treatment of 44 cases of idiopathic neonatal hypoglycemia.

METHODS

Chemical methods

The determination of blood glucose has been described (20). Hemoglobin and serum calcium were measured with routine clinical laboratory procedures. The urinary excretion of estriol was determined according to the method of Brow (4).

Therapeutic trial

The screening procedures for the detection of hypoglycemia have been described (20). Therapeutic measures were initiated, if the glucose value in the capillary blood of an infant was found to be 20 mg/100 ml or less, regardless of the symptoms. A catheter was inserted into the umbilical vein, blood sample was draw and therapeutic test with 1.0–1.5 grams of glucose was performed. Thereafter, an intravenous infusion of 10% glucose at the rate of 80–100 ml/kg body weight per day was started. In addition, every other hypoglycemic patient, in the order of admission, received 5 mg of hydrocortisone (Solu-Cortef, Upjohn) intramuscularly three times a day During therapy as well as for two days after its cessation the blood glucose levels were monitored at least five times day The glucose infusion was tapered and stopped after the levels remained above 40 mg/100 ml, but not until after minimum of 24 hours. Hydrocortisone was administered for at least three days and then gradually stopped, if normoglycemia had been achieved.

For the evaluation of the immediate effect of treatment, the duration of hypoglycemia was chosen as the criterion. Because practical method for the continuous recording of blood glucose levels is not available, only rough approximation of the actual duration, based on the five or more daily determinations, could be made The time from the initiation of treatment to the last blood glucose value of 40 mg/100 ml or less was, therefore, used as the basis for comparisons. Reactive hypoglycemia and possible occasional low values after the establishment of satisfactory general level have not been taken into account in this evaluation.

Definitions

Hypoglycemia was considered to be *significant*, if the glucose level was found to be 20 mg 100 ml or less in at least two separate blood samples.

Hypoglycemia was defined as *symptomatic* if the low blood glucose level was associated with symptoms that ere considerably or completely relieved within minutes or few hours after the intravenous injection of 1.0–1.5 grams of glucose.

Table 1 *Obstetric background of 44 cases of neonatal hypoglycemia (43 pregnancies)*

Obstetric history of mother		Current pregnancy		Deliver	
Primiparous	23	Duration, weeks		Norma	19
Multiparous	20	32 or less		C mpl	11
Earlier pregnancies normal	10	32–33	3	Bre	6
Abortions or stillbirths	10	34–35	8	Ind	3
		36–37	14	Va	1
		38–41	14	M	1
		Over 41	2	Caesar	13
		Complications	34	Elect	6
		Toxemia	29	Fetal asph	5
		Antepartum hemorrhage	5	Dystocia	
		Twin pregnancy	3	Fetal asphyxia dur	
		Uncomplicated	9	labor and de	1

Low birth weight for gestation, dysmaturity and *"small for dates"* were regarded as synonymous terms referring to infants, whose birth weights were below the 10th percentile for gestation. For the assessment of this relationship, intrauterine growth curves based on a Finnish obstetric population (24) were used.

Repeated diastolic blood pressure readings above 90 mmHg and/or albuminuria were considered as criteria for *toxemia of pregnancy*.

Meconium staining of the amniotic fluid, distinct irregularities in the fetal heart rate and acidosis in fetal capillary blood obtained during delivery (21) were interpreted as signs of *fetal asphyxia*.

CASE MATERIAL

Obstetric background (Table 1)

Of the 43 mothers 23 were primiparae. Ten of the 20 multiparous mothers had experienced previous obstetric accidents.

The current pregnancy was uneventful in only nine cases. Toxemia was by far the most common complication, present in two thirds of all pregnancies. In 11 of the toxemic mothers the estriol excretion had been monitored during the last trimester in an attempt to evaluate the function of the fetoplacental unit. In seven of these, the excretion was repeatedly found to be less than 5 mg/24 hours, in comparison with the normal values of 15–30 mg/24 hours in late pregnancy (11). In three mothers a significant fall in the estriol excretion to values below 10 mg/24 hours occurred during the last week before delivery whereas in only one case the excreted amount remained normal.

Four of the patients were twins. One of them was the smaller of a discordant (2) pair, another the larger of a pair with approximately similar birth weights, and of the third pair also with similar birth weights, both members became hypoglycemic.

Most of the infants were born at or near term but 13 were more than four weeks pre-term. Nineteen mothers had a normal vaginal delivery whereas the others experienced complications or were delivered operatively. Fetal asphyxia was noted in 16 cases, in 5 of which the labor was terminated by cesarean section.

Neonatal features (Table 2)

Although complications during pregnancy or delivery were seen in most cases, only a few of the infants were born in poor condition. An Apgar score of 3 or less at five minutes of age was recorded in seven cases, four of which required endotracheal intubation and assisted ventilation. This postnatal asphyxia subsided by the age of 60 minutes in all except one, who had the aspiration syndrome and required assisted ventilation for three days. In two other cases respiratory distress developed and ventilation had to be resumed during the first day of life.

Thirty of the infants were males and 14 were females. The birth weight was 2500 grams or less in 29 cases, and dysmaturity was evident in 3 cases. Only four infants had a birth weight exceeding the 50th percentile for gestation.

Sixteen infants were hospitalized for prematurity alone whereas the others had more specific symptoms or signs as indications for referral. Thirty seven arrived in the hospital during the first day of life, 11 of them with the diagnosis of hypoglycemia. Seven patients were admitted after the age of 24 hours, and in only two of them hypoglycemia had been demonstrated, although all

Table 2. *Neonatal features of 44 cases of hypoglycemia*

Sex	
Males	30
Females	14
Birth weight g	
1500 or less	12
1501 2000	11
2001 2500	6
2501 3000	9
3001 3500	6
Dysmaturity	32
Condition at birth	
Apgar score	
0– 3	7
4– 6	14
7 10	23
Age at admission, hrs	
6 or less	26
6–24	11
24–48	3
48–72	4
Causes of admission	
Prematurity	16
Asphyxia	6
Cyanotic attacks	5
Convulsions	2
Congenital anomalies	2
Hypoglycemia	13

had symptoms. Two infants had received peroral glucose and hydrocortisone because of the hypoglycemia elsewhere.

Hypoglycemia (Table 3)

The first subnormal blood glucose value was measured at the age of six hours or less in 16 infants, and during the first day of life in 34. Only three infants were completely asymptomatic at the time of measurement, whereas in the others variable abnormal symptoms or signs were observed. The therapeutic test was definitely positive in only 12 of these 31 patients, but in 4 cases a coexisting disorder dominated the clinical picture so completely that an alleviation of possible minor symptoms of hypoglycemia would have been impossible to detect.

In ten infants hypoglycemia was demonstrated after the age of 24 hours. Eight of them were admitted because of symptoms clearly attributable to low blood glucose, the diagnosis was made within hours after admission and the response to glucose was prompt. Two were nursed in the premature ward since four hours of age and they developed hypoglycemia during the third day of life after several borderline values. They had mild tremulousness as the only symptom and they responded to glucose only gradually.

The symptoms were nonspecific but, nevertheless, suggestive in the majority of cases. Jitteriness, tremors, respiratory irregularities and an exaggerated Moro reflex were the dominant features during the first day of life. Thirteen infants had convulsions, which seldom occurred before the second or third day. Only three convulsed during the first day of life one of these had cerebral hemorrhage, the other two had been severely asphyxiated but no definite signs of hemorrhage were detected. In many cases the hypoglycemic symptoms were very mild particularly in the more premature infants and during the first hours of life.

In five cases a venous blood sample was not obtained. In 35 infants the glucose concentration in umbilical vein blood was below 20 mg/100 ml and in agreement with the capillary value, whereas in four cases the venous value was above 20 mg/100 ml. This was considered to result from the admixture of hepatic vein blood with a somewhat

Table 3 *Features of hypoglycemia in 44 newborn patients*

Age at diagnosis, hrs		Symptoms		Duration, hrs (from first to last value of 20 mg/100 ml or less)	
6 or less	16	Tremors, jitteriness	33	24 or less	24
6–24	18	Apnea, cyanosis, tachypnea	22	24–48	5
24–48	1	Lethargy	9	48–72	7
48–72	7	Convulsions	13	72–96	3
Over 72	2	N symptoms	3	96–120	3
		Symptoms masked"	4	Over 120	2
		Therapeutic test positive	20		

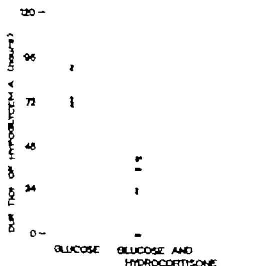


Fig. 1 Duration of hypoglycemia, from the initiation of treatment to the last glucose value of 40 mg/100 ml or less, in patients treated with glucose infusion alone or with glucose infusion and hydrocortisone

higher glucose content; this possibility existed as the position of the tip of the catheter was not ascertained.

Disorders associated with hypoglycemia

Primary cerebral damage was verified in two cases. One had congenital toxoplasmosis and developed hydrocephalus, which was diagnosed at the age of one month. The other had cerebral hemorrhage, verified at autopsy at the age of eight months, after the severely retarded child had died of a second cerebral hemorrhage occurring during a course of ACTH-therapy for infantile spasms. Primary cerebral damage was suspected in two other infants because of increased protein in the cerebrospinal fluid. Lumbar puncture was performed in 11 other cases, and the findings were normal.

Pulmonary disease of such a severity as to necessitate assisted ventilation was present in three patients. One of them had the idiopathic respiratory distress syndrome and two had the aspiration syndrome.

Serum calcium levels below 7 mg/100 ml were measured in seven infants, one of which even had values below 6 mg/100 ml. Peroral calcium gluconate was given to these patients, although hypocalcemic symptoms were not observed. Polycythemia with a hemoglobin concentration of 25 g/100 ml or more was present in two patients.

D[illegible]rse congenital anomalies [illegible] seen in eight cases. These included one infa[illegible] [illegible]congenital heart disease, one with esopha[illegible] [illegible], two with hypospadia, two with m[illegible] [illegible]omalies and two with mongolism.

TREATMENT

The immediate object of ther[illegible] [illegible] attainment of normal blood glucose [illegible] a quickly as possible. The results of treatmen[illegible] [illegible]h glucose infusion alone or in combination [illegible] hydrocortisone administration were comp[illegible] [illegible] basis of the duration of hypoglycemia. [illegible] [illegible]1 infants originally treated with gluco[illegible] were excluded from the evaluation, bec[illegible] [illegible] [illegible]sistent symptoms and low blood glucose l[illegible] after 24 hours of infusion were considered [illegible] necessitate hydrocortisone administration. Three of the 21 infants belonging to the hydrocortisone group were similarly excluded because of deviations from the therapeutic regime. Additional exclusions were the two infants treated before admission.

The results of the two forms of therapy are compared in Fig. 1. The mean duration of hypoglycemia in the group treated with glucose and hydrocortisone was significantly shorter than in the group receiving glucose alone. This conclusion is not invalidated by the exclusions mentioned above. On the contrary, as they were among the most difficult cases of the glucose group, the difference in results would probably have been even greater if the therapeutic regime had been strictly adhered to at the risk of cerebral damage.

Prolonged infusion of glucose without saline addition was the probable cause of marked edema formation in eight patients, two of which even required digitalization and diuretics because of impending pulmonary edema. After dilute saline has been included in the glucose infusion, only one case with mild edema has been seen. Reactive hypoglycemia with blood glucose values below 20 mg/100 ml occurred in seven patients, in which the infusion had infiltrated or was stopped too abruptly, but none of these incidents was associated with symptoms. No other complications of treatment were observed.

OUTCOME

Due to the shortness of the follow-up period, only a preliminary evaluation of the prognosis is pos-

sible. So far severe mental retardation with spasticity has been observed in three cases, all of which have infantile spasms with typical EEG-findings as well. One of these had convulsions when first admitted at the age of two days, and was classified as symptomatic hypoglycemia at that time On the other hand, the two others were three hours old on first admission, and the mild symptoms observed at that time were uninfluenced by glucose. The mental retardation was obvious before the age of six months in all three cases Another retarded infant with primary cerebral damage died at the age of eight months, but the rest of the patients seem to be developing normally after 4–?6 months of observation. Recurrence of hypoglycemia has not been observed in any of the cases so far

DISCUSSION

Our experience on patients with neonatal hypoglycemia agrees in several respects with the views of other workers. The sex ratio of 2.1 1 in favor of the males is identical with that presented by Cornblath & Schwartz (9) on the basis of reports in the literature. The greater susceptibility of the males *evidently* depends on other etiological factors besides intrauterine malnutrition, as among the dysmature infants surveyed in this hospital (20) the male female ratio was only 1.2 1

Dysmaturity was more dominant in the present 3. out of 44 cases than in several other reports (9 15) Toxemia is a common predisposing cause of dysmaturity and, in our experience, the excretion of estriol seems to reflect the impairment of placental function in toxemia, provided that the fetus remains viable. Therefore, the possible development of hypoglycemia should be anticipated in infants of mothers, who excrete subnormal amounts of estriol in late pregnancy Conflicting views on this question have been expressed (3 25)

Our case material is based on systematic screening of all admissions and not on an analysis of symptomatic cases. This is evidently the reason for the early average age of diagnosis in comparison with other reports (5 9 13). In the typical case, it seems that the blood glucose level begins to fall shortly after birth. During the first hours of hypoglycemia symptoms are few or absent, but gradually jitteriness and respiratory irregularities appear at first not responding to glucose. As the symptoms become more severe, they are more obviously alleviated by an injection of glucose, and by the third day of life convulsions are common in the untreated case. Permanent normoglycemia is usually established by the age of one week.

The "therapeutic test evaluated on the basis of the present series, was not as satisfactory as expected from the experience of others (9 16). In symptomatic cases admitted during the second and third days of life the response was unequivocal but the majority of our patients, admitted before the age of 24 hours with subtle symptoms, were recorded as negative. Two of the three infants, in which mental retardation has later been observed, were not classified as symptomatic at the initiation of treatment. Neither clinical evidence nor laboratory studies suggested the presence of other disorders to account for the several failures to respond to glucose. Thus, the value of the therapeutic test seems to be limited in the early as well as in the more advanced stages of the disease (10)

Most investigators interested in neonatal disorders of carbohydrate metabolism have concentrated their attention on symptomatic hypoglycemia. *Asymptomatic infants with comparable* blood glucose levels have often been thought only to represent one extreme of physiological variation and not worthy of serious consideration or treatment (7 13 16), although opposing views have also been expressed (6).

For a number of reasons, we think an expectant attitude to be both unjustified and potentially dangerous to the infant. Firstly a low birth weight neonate, especially if born well before term, is probably incapable of reacting to harmfully low glucose supply as promptly and distinctly as a mature newborn or older infant. Secondly the early symptoms are usually mild and quite nonspecific, and their detection depends on the experience of the observer and the closeness of supervision. In practice, neither factor can be confidently relied upon, when the distinct possibility of cerebral damage is at stake. The same *limitations naturally apply to the interpretation* of the therapeutic test in many cases.

The usually accepted lower limit of normoglycemia, 20 mg/100 ml is based on statistical and empirical considerations (9). Actually the critical level from the point of view of cerebral glucose supply is unknown and probably variable, because

of the interplay of other factors. The same can be said of the critical duration of the hypoglycemia, but extensive pathological changes in the nervous system have been described after less than two days of intermittent hypoglycemia (1). This uncertainty makes it still more difficult to see the justification in waiting for symptoms to appear before treatment is initiated.

For the reasons stated, we believe that all infants with significant hypoglycemia should receive immediate and sufficiently intensive therapy. The value of corticosteroids seems definite, although the mechanism of the hyperglycemic effect is unclear. Increased hepatic production of glucose, either through provision of substrate or induction of enzymes for gluconeogenesis, is suggested by the *results of experiments on glucocorticoid action* (26), and such a mechanism would be in agreement with the time-course of the effect observed in our series.

SUMMARY

Forty-four newborn infants with significant hypoglycemia, i.e. with two or more true blood glucose values of 20 mg/100 ml or less, have been studied. Two thirds of the patients were males, and a similar proportion had low birth weight for gestation, mostly associated with maternal toxemia.

Hypoglycemia was diagnosed during the first day of life in 34 cases. Only three infants were asymptomatic, whereas the others exhibited various nonspecific symptoms, which generally were more severe in patients aged two or three days. A therapeutic test with glucose was positive in only 20 infants, and mostly negative before 24 hours of age. The hypoglycemia was transient in all cases.

Mental retardation with spasticity and infantile spasms has developed in four infants by the age of six months, and one of them died at the age of eight months. The others appear normal after 4–16 months of observation.

A significant effect of hydrocortisone in shortening the duration of hypoglycemia was demonstrated.

On the basis of experience with the patients reported, it is suggested that all infants with significant hypoglycemia should be efficiently treated, regardless of symptomatology.

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CASE REPORT

AN INHERITED CHROMOSOME ABERRATION IN A GIRL WITH SI OF DE LANGE SYNDROME

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The de Lange's syndrome (dLs) originally described by Cornelia de Lange in 1933 (4) is characterized by mental retardation, short stature and a cluster of minor malformations, the facial appearance being most characteristic (see Table 1) (13 16). The development of clinical cytogenetics caused a renewed interest in this syndrome. Sixteen cases with chromosomal aberrations have been found in one hundred cases of dLs where chromosomal studies have been performed. The chromosomal aberrations have been of different structural kinds (1 3 5 6, 7 10 15) and in some patients a supernumerary fragment has been observed (2, 11) In addition a patient with some signs of dLs and an extra chromosomal fragment has been reported (12) Thus, most of the patients have normal chromosomes and a uniform abnormality has not been verified in the rest. It is now evident that an apparently normal karyotype is the expected finding in a patient with dLs. A structural aberration not visible in the microscope can perhaps explain the phenotype of dLs (13) It has also been stated that phenotypes which resemble that of dLs can perhaps be produced by unspecific chromosomal imbalance (13). This paper describes a girl of normal stature who has many of the signs of dLs and an inherited chromosome aberration.

CASE REPORT

The proposita, girl 8 years of age was admitted to hospital because of convulsions.

Family history

The parents were physically and mentally normal, the father having confluent bushy eyebrows. There was no known consanguinity Two older sisters were also phenotypically normal. A brother, born one year befor th proposita, died at the age of 1 days. He the product of full-term pregnancy his birth eight being 1660 g and length 42 cm. Autopsy showed skin and bone defects biparietally micrognathia, cleft palate, hypospadia, and synechia between the third and fourth digits f the right foot. Aortic stenosis, idely patent ductus arteriosus, and ventricular septal defect were also demonstrated

Past history

The birth history showed spontaneous delivery at 39 weeks gestational age, the mother being 33 and the father 36 years old. The following measurements were noted at birth. weight 3450 g, length 51 cm, and head circumference 33 cm. Ther was lanugolike hirsutism over the forehead (Fig. 1), back, and extremities. A coccygeal malformation, with the coccyx deviated backwards, and xtra digits on the fifth digit of the left hand and foot were present. There was mild respiratory distress after birth. A praecordial systolic murmur was noted after the first week of life and repeated cyanotic spells took place. She had difficulty in feeding and did not gain in weight From the fifth month of life, convulsive seizures occurred. The girl as found to be mentally retarded by the first year of life. She could sit at the age of 18 months and *began to walk at the age of three years. Speech development* was retarded, she could only repeat simple words at the age of five years. From the first year of life she suffered from repeated respiratory tract infections.

Present condition (see Table I)

On admission, physical examination showed a well-nourished girl 125 cm in length (75th percentile) and eight 23 kg (50th percentile). The head was microcephalic: circumference 49 cm (below 3rd percentile), cephalic index 0.79 The appearance of the face is shown in Fig. 1. The forehead was low and covered with lanugolike hair Eyebrows were bushy and confluent and eyelashes long and curved. The nose was small with flat bridge and anteverted nostrils. The upper lip was thin and the distance between it and the nose was lengthened. The palate was high and narrow and the teeth small and irregular The ears were normal but somewhat low

Table 1 *Clinical characteristics of de Lange syndrome*
Clinical characteristics of the proposita with italic type

General	Facial appearance	Central nervous system	Skeletal system
Short stature	*Confluent bushy eyebrows*	*Mental retardation*	Skeletal changes arms and hands
Hirsutism	*Long curved eyelashes*	*Microcephaly*	Proximally placed thumbs
Low-pitched cry	*Short upturned nose*		Syndactylism between 2-3 toes
	Elongated philtrum		
	Thin upper lip		
	Low-set ears		

In addition, congenital heart defects and other internal malformations have been noted.

set. The thorax was well developed, and there was no hirsutism. The extension at the elbow joints was normal. The fingers were short and tapering with a normal number of flexion creases. The palmar creases and the implantation of the thumbs were normal. The feet appeared dysplastic. There was a systolic murmur over the left second intercostal space and the diagnosis of an infundibular pulmonary stenosis was confirmed after heart catheterization and angiography. The speech was indistinct. According to psychological test (Terman–Merrill) the I Q was 44.

Radiological studies. Radiographs of the skull, extremities, and pelvis showed no abnormalities. A chest X-ray revealed that the 12th ribs and the corresponding vertebral body were missing.

Laboratory studies. The activity of the blood cell galactose-1-phosphate uridyl transferase was normal, 30.1 units/g Hb. Other blood tests, including enzyme and electrolyte determinations, were also normal. Electrophoresis of the cerebrospinal fluid showed an increase of the alpha and beta globulin fractions as a sign of cortical degeneration. This agrees well with the echoencephalography which showed signs of cortical atrophy. A pathological electroencephalogram with bilateral paroxysmal and slow activity was also found.

DERMATOGLYPHIC STUDIES

The dermatoglyphs of the proposita, her parents and her sisters were examined. The palm prints of the proposita showed a strikingly lower total ridge count and a strikingly higher maximal *atd* angle than those of all her relatives (Table 2). In addition, there was a syndactylous pattern between the second and third toes of the feet. These findings fit rather well with the findings in the cases of dLs reported by Smith (17).

CYTOGENETIC STUDIES

Chromosomal studies of the proposita, her parents, siblings, maternal grandparents, and maternal sister



Fig. 1. The proposita at age 5 months and 8 years.

Table 2. *Dermatoglyphic patterns on hands of proposita and [illegible] relatives*

A arch; R radial loop; U = ulnar loop; W = whorl

	Finger tips												
	Left					Right					Total ridge count	[illegible]	[illegible]
	V	IV	III	II	I	I	II	III	IV	V			
Proposita	U	U	A	A	U	U	A	U	U	U	[illegible]		[illegible]
Father	U	W	U	W	W	W	W	A	U	U	145	[illegible]	[illegible]
Mother	U	U	U	U	W	W	W	U	W	U	165		[illegible]
Sister, 16 y	U	U	U	W	U	W	U	W	U	U	163		
Sister, 13 y	U	W	U	R	U	W	R	U	W	U	137	41	

were made on leucocyte cultures by a modification of the method of Moorhead *et al.* (14). The proposita showed the modal chromosome number of 46. An abnormal karyotype, however, was consistently present. One of the group B chromosomes was replaced by a chromosome similar to a number 3 chromosome (Fig. 2). In some metaphase plates, this chromosome could not be distinguished from the number 3 chromosomes, while in others, two identical number 3 chromosomes were found together with a somewhat longer and more asymmetric chromosome. The karyotypes of the mother and the two sisters were also abnormal. T[illegible] aberration as in the proposita was found, [illegible]tion, the long arm of one of the group D [illegible]somes was shorter than normal (Fig. 3). In Fig. [illegible] the group D chromosomes from six metaphase plates of the proposita, her mother and one [illegible] her sisters are compared. The father, the maternal grandparents, and the maternal sister had normal chromosomes (Fig. 5).

The simplest interpretation of the chromosome

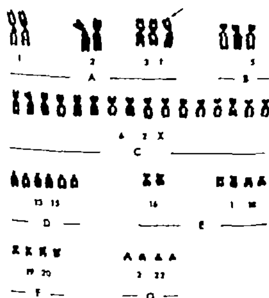


Fig. 2. The karyotype of the proposita demonstrating partial trisomy D. One of the group B chromosomes is replaced by chromosome (arrow) similar to No. 3 chromosome (= translocation-chromosome). For further explanation see text.

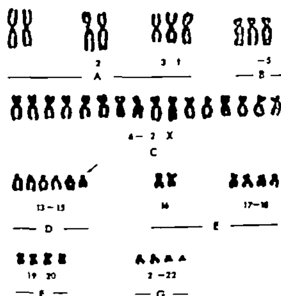


Fig. 3. The karyotype of the mother. This karyotype is identical with those of the two sisters of the proposita. One of the group B chromosomes is replaced by chromosome (arrow) similar to No. 3 chromosome and in addition the long arm of one of the group D chromosomes is shorter than normal (= translocation-chromosome). For further explanation see text.

a b c

Fig. 4. The group D chromosomes from six cells of the proposita (a), the mother (b), and one of the sisters of the proposita ( ). The chromosomes of the mother and the sister demonstrate a D chromosome (arrow) with a long arm somewhat shorter than normal.

findings in the mother and her three daughters, taken in association with the fact that the mother two of her daughters appeared normal phenotypically is that there has been a reciprocal interchange of unequal portions of arms of one of the group B chromosomes and one of the group D chromosomes. A portion of the long arm of a group D chromosome seems to be attached to the short arm of a group B chromosome. The morphology of the translocation-chromosome is, however not fully explained by this interpretation. The long arm of the translocation-chromosome usually seems to be somewhat shorter than that of a group B chromosome. In addition one of the group C chromosomes was hard to pair in some cells. Thus a more complicated rearrangement, in which more than two chromosomes are engaged, cannot be excluded. Autoradiographic investigations are in progress. Examination of buccal smears of the mother the sisters, and the proposita showed sex chromatin bodies of normal size.

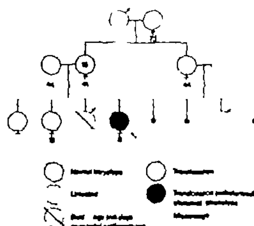


Fig. 5. Pedigree showing the inheritance of the translocation. The proposita is indicated by an arrow.

The karyotype of the mother and the two phenotypically normal daughters seem to be balanced, whereas the proposita, with normal group D chromosomes, probably has an unbalanced karyotype with extra group D material. The proposita, however has no signs of the trisomy D syndrome.

DISCUSSION

The clinical diagnosis of dLs is based on a pattern of signs and malformations, none of which are specific (Table 1). The suspicion is raised by the characteristic facial appearance of these patients. The same statements could be made concerning patients with Down's syndrome. In Down's syndrome there is a high incidence of clinical variability (8) whereas Ptacek *et al.* (16) state that infants with dLs have a more uniform appearance than patients of any other known syndrome. As the clinical variability in dLs is said to be low the diagnosis of dLs cannot be stated in our case, where one of the most important signs of dLs, the short stature, is missing. The present child had also a normal galactose 1-phosphate uridyl transferase activity. Hall & Dahlqvist (9) reported a somewhat high activity of this enzyme in two adult cases of dLs. McArthur & Edwards (13) state that many of the published cases with dLs are misdiagnosed. The misdiagnosed cases closely resemble this distinct syndrome, which can perhaps be simulated by other generalized disorders, particularly those related to chromosomal imbalance. The present case with a phenotype resembling that of dLs, in combination with a chromosome aberration, could possibly exemplify this statement. The diagnosis of dLs, which motivated the chromosomal investigation, was disqualified after a renewed investigation of the proposita in which the other family members were included. The bushy eyebrows of the normal father suggested that this sign in the proposita is a family characteristic. This, together with the normal stature of the girl, made the diagnosis of dLs unlikely. The mental retardation and the clinical features of the girl, which are not typical of any known syndrome, must be considered an unspecific general dysplasia caused by an unbalanced chromosomal aberration. A parallell phenomenon has been described by Stalder *et al* (18) in a family with balanced D/C-translocation carriers and unbalanced offspring—a partial D trisomy.

A strict definition of de Lange syndrome and further investigations of the clinical variability would be valuable. A chromosomal aberration not detectable in the microscope, has been suggested as the cause of the dLs syndrome (13). If this suggestion is correct, a wide clinical variability would be expected according the xperience from other chromosomal syndr

SUMMAR

A mentally retarded girl with of de Lange syndrome is described a has a chromosomal aberration, u he un-affected mother One chromo p B and one of group D are engag tion, and the possibility of being involved in this rearra gem nt excluded. The proposita has a par l I The variability of de Lange syndrom

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CASE REPORT

FREEMAN SHELDON'S SYNDROME, CRANIO-CARPO-TARSAL D

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Two children who were not related and who obviously constituted a syndrome of their own, one that had not been previously described, were reported by Freeman & Sheldon in 1938 (1) A further case of the same type was encountered by Otto in 1953 (3). No other descriptions of this syndrome have been found in the literature. A fourth case is now presented here.

CASE REPORT

The child, a boy was the first of a healthy mother with no known family history of anomalies. Delivery was by breech presentation. The pregnancy had been normal and the mother had not taken drugs during it. The infant's birth weight was 3930 g. He was first awarded an Apgar score of 6, after an hour 10

The bilateral pes equinovarus was corrected operatively and the child's mouth was widened by plastic surgery After prolonged, recurrent respiratory tract infections the child had cardiac arrest at the age of one year 1.5 months and died in spite of efforts at resuscitation.

The anomalies established in the patient and the pathologic findings are summarized in the following (see also Table 1)

Skull. Longish, laterally flattened skull although the sutures were not fused. In the soft tissues of the occiput an inaccurately defined prominence which gradually flattened with age. The anterior cerebral fossa was steeper than normal (Figs. 3 and 4).

Face There was pronounced enophtalmos and strabismus and mild hypertelorism. The ... were of normal size however and the ocu... fundi also normal The nose was rather small and the philtrum of considerable length. The orbicularis oris muscle formed a partly scar-like dense strand around the exceedingly small mouth The facial bones as a whole were hypoplastic compared with the skull. The maxilla was markedly narrowed so that the palate was merely a groove like formation (Figs. 1 3 and 4)

Jaw The mandible was short, narrowed and lacked normal angle formation. The jaw was small, with two vertical symmetrical grooves on the skin (Figs. 1 3 and 4)

Thorax: Narrow thorax, deeper than normal in the sagittal direction. Partial right bundle branch block in the ECG

Abdomen and genitals: On the left inguinal hernia, on the right undescended testis (inguinal canal).

Upper extremities: The wrists showed only very slight, but the fingers distinct ulnar deviation, the thumbs were partly digitalised. The fingers showed distinct extension deficiency in the metacarpophalangeal and proximalinterphalangeal joints. A scar-like flexion contracture was seen on the volar side of the thumb. The bony structure was normal (Fig. 2).

Lower extremities There was severe bilateral pes equinovarus.

The blood values, urine, spinal fluid and immunoelectrophoresis were within the normal range.

The patient was sex chromatine negative. His chromosomes were analyzed from peripheral leu-

Table 1 *Comparison between the cases described in the literature and the present case*

Feature	No. of case Freeman & Sheldon I	II	Otto III	Our case IV
Parity of child	I	I	I	I
Sex	M	F	F	M
Birth weight, g	1600	2040	[illegible]850	3930
Difficulty in drinking	+		−	+
Edema				
of occiput	+		−	+
of forehead	+		−	−
Large head in relation to face			+	+
Prominent cheeks				
Enophtalmos	+		+	+
Strabismus	+		+	+
Epicanthus				+
Hypertelorism				
Small nose	+			±
Long philtrum				
Small mouth				
Grooves in the jaw	[b]		+	
Ulnar deviation of the hands	+	+		
Finger contractures	−	±		+
Pes equinovarus				+
Spina bifida occulta	−	−	−	−
Retarded intellectual development	±			

Judging by the photographs.

[b] Freeman & Sheldon stated that there is an unusually well-marked dimple on the mid-point of the chin. Otto paid no attention to the jaw. Photographs of the cases of both authors reveal distinct grooves.

Slight deviation of the wrists but distinct deviation of the fingers in the present case.

I Otto's case, pes equinovarus on the left, "Knickhackenfuss" on the right.



Fig 1 The patient at the age of weeks. Note the deep-lying eyes, slight hypertelorism and epicanthus, long philtrum, small mouth and vertical symmetrical grooves in the mandible.



Fig 2 Left hand at the age of weeks. Clinodactylism, partial digitalisation of the thumb and ulnar deviation of the fingers visible.

cytes, cultivated according to a modified method of Moorhead et al. (2) Fifty nine of the 65 analyzed cells had 46 chromosomes while 6 cells were lacking a chromosome at a random way. The sex chromosomes were XY. The patient and his mother had an abnormally long paracentric secondary constriction in one of the C-group chromosomes, to be described in another connection. Otherwise the karyotypes of the patient and his both parents were considered normal.

It was found when the patient was one year old that hearing was distinctly and vision possibly impaired, but pain sensation was normal. Psychologic testing showed distinct retardation of intellectual development. It was regarded, however, mostly secondary due to the numerous anomalies.

DISCUSSION

The photographs alone show convincingly enough that the three cases previously described in the literature and the present case belong to the same syndrome. The degree of severity and the number and nature of the anomalous features varied considerably. The present case was definitely the most severe.

All the cases described were firstborn, but as shown by the present patient the syndrome does not necessarily seem to be associated with prematurity as was assumed by Freeman & Sheldon



Fig. 3. Roentgenogram of the skull, lateral view, at the age of 1 year. Note the steep anterior cerebral fossa, small size of facial bones in relation to the skull, straight lower jaw and position of the teeth.



Fig. 4. Anteroposterior roentgenogram of the skull at the age of 1 year. The skull is flattened laterally. Note the narrowness of the mandible and maxilla (teeth).

(1). Although many of the anomalies of the present case resembled cases of trisomia reported earlier in the literature, the chromosome study showed nothing to support this.

None of the previous authors paid any appreciable attention to the vertical folds of the skin of the jaw, though they are clearly visible in the photographs. Both in location and shape they resembled the lateral sulci of the lower lip, which occur in the embryo in the 7.5–12.5 mm stage (6–7 weeks) but normally subsequently disappear (5). The sinuses which sometimes appear in the lower lip, are assumed to be remnants of the cranial ends of lateral sulci (4, 5). The grooves in the present patient correspond in their location most closely to the medial and distal parts of the lateral sulci. Histologic examination of the fold showed that it was lined by [illegible] epithelium, like the sinuses of the lower lip, and nothing suggestive of a cleft [illegible]

in the underlying tissues. This, too, argues in favour of the hypothesis advanced in the foregoing, that the folds originate from the lateral sulci.

SUMMARY

A patient with Freeman–Sheldon's syndrome (cranio-carpo-tarsal dystrophy), obviously the fourth case of its kind to be published, is described. No significant chromosome anomalies were demonstrated. Attention is drawn to the vertical grooves on the patient's jaw and lower lip which may be remnants of the so-called lateral sulci established in the embryo in a certain phase of development.

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Key words: Cranio-carpo-tarsal dystrophy, Freeman-Sheldon's syndrome

CASE REPORT

BENIGN FAMILIAL NEONATAL CONVULSIONS

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Convulsions in the neonatal period are rather common in brain damage of various origin. Another well-defined group with early convulsions comprises the metabolic diseases with various enzyme defects, but this group is relatively small (2, 8, 9 11). A common feature in these diseases is that the metabolic dysfunction usually leads to serious destruction of the brain parenchyma with mental deterioration as the main defect.

The present report concerns a family where 14 members in 5 generations had frequent convulsions during the first weeks of life but with a favourable outcome. The youngest siblings in the last generation were observed by the authors and are described as cases 1 and 2, see pedigree. The main findings in the other family members are grouped together in Table 1

CASE REPORTS

Case 1

M.P Girl, born 1962. Pregnancy and delivery were normal. Birthweight 4200 g She had bilateral congenital dislocation of the hip. From the third to the 23rd day of life she had 118 observed general convulsions of short duration and 40 observed attacks of cyanosis with the maximum frequency on the fifth day of life, when 38 attacks were observed. The girl was quite well between the convulsions and showed no neurologic abnormalities. Routine blood examinations (haemoglobin level, red and white blood cell count) were normal. Cerebrospinal fluid was not examined. Plasmaelectrolytes were normal. Blood sugar was 30 mg/100 ml at the fifth day of life. Repeated morning values and blood sugar curve throughout 24 hours were normal. Owing to the borderline blood sugar value 30 mg/100 ml, glucose in 5.5% solution and corticotrophin (Actos®) were given without effect on the convulsions. EEG tracings at 3 weeks of age and at one month were normal Phenobarbital injections 0.01 given repeatedly without effect Pyridoxine treatment given with large doses perorally and intramuscularly without any effect on convulsions (Fig 2)

From the age of 23 days to 3 months the girl had no convulsions. At the age of 3 months she was admitted at hospital again because of general convulsions and acute infection of the middle ear with fever During the 10 day stay in the hospital no convulsions were observed. The patient was well apart from the ear-throat infection which was treated with myringotomia and antibiotics.

Blood and urine examinations were normal Blood sugar was normal. Serum calcium and phosphorus were normal EEG during sleep at four months of age showed epileptogenic activity within the left occipital quadrant. At five years of age the girl is reported to be quite well and normally developed and has not had further convulsions.

Case 2

M.P Boy born 1967 Pregnancy and delivery were normal. Admitted to Children Department directly after birth because of the risk for convulsions in the family history From the third to the ninth day of life 21 general convulsions lasting i/₂–3 minutes and 36 attacks of cyanosis were observed. Between the attacks the boy was quite well and no neurological abnormalities were noted. Routine blood and urine examinations were normal. Cerebrospinal fluid was normal (protein content and cell count). Blood sugar was normal. Serum calcium 4.9–4.2 mEq/l. Serum phosphorus 6.5 mg/100 ml. Serum magnesium 1.5 mEq/l. Serum copper and ceruloplasmin normal. Tryptophan load test normal. "Metabolic screening" of the urine was performed. Albustix, Clinistix, Phenistix, Almén's reaction (revealing reducing substances) and the chromatographic pattern of the amino acids on urine specimen were all normal. EEG at 5 days of age (during convulsions): Left sided abnormality without definite focus. EEG at 10 days of age: Normal curve. Treatment: On 3rd to 7th day phenobarbital 15 mg daily on 3rd to 6th day pyridoxin 80 mg daily and 7th to 13th day 120 mg pyridoxin daily

in the underlying tissues. This, too argues in favour of the hypothesis advanced in the foregoing, that the folds originate from the lateral sulci.

SUMMARY

A patient with Freeman–Sheldon's syndrome (cranio-carpo-tarsal dystrophy), obviously the fourth case of its kind to be published, is described. No significant chromosome anomalies were demonstrated. Attention is drawn to the vertical grooves on the patient's jaw and lower lip which may be remnants of the so-called lateral sulci established in the embryo in a certain phase of development.

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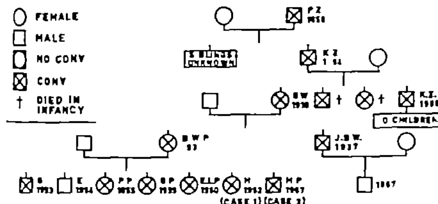


Fig 1 Peu

There was no notable effect of the treatment. At control len 4 months old the boy was quite ell and had had no convulsions since he was discharged from the hospital without treatment. The psychomotor development was quite normal.

These two siblings had high inherited potential for neonatal convulsions. They were number 6 and 7 of 7 siblings of whom number 1, 3 4 and 5 also had had neonatal convulsions and had been treated in the neonatal period at the Children Department, Malmö Allmänna Sjukhus. The same is true of the mother of these children and her only brother. According to the family history obtained at interview ith the maternal grandmother of case 1 and 2 (G. W no. 9 in Table 1) another 6 members of the family 3 generations back also had had convulsions in early life See pedigree Fig. 1 and Table 1

DISCUSSION

The clinical picture in all these cases has been very similar After a normal delivery general convulsions and attacks of cyanosis ha e sta the 3rd day of life The period of con ul has varied between 3 days and about 3 w k During this period seizures ha e been fairly frequent. After this initial disease period 3 female members have had isolated short con uls ons together with febrile illness and male members have had sporadic seizures up to 10 years f age. The motor and mental development were in the 12 cases alive quite normal. 2 patients died n their first year of life, but this was in the third generation born in the beginning of the 20th century and the cause of death is obscure. These children are said to have died from convulsions but it is quite feasible that these convulsions have appeared during an infective disease which may have been the direct cause of death.

All the patients were born after a normal pregnancy and all the deliveries were normal except that of J B. W who weighed 5600 g and was delivered by forceps. This delivery took place at home and was said to be easy (noted in the record when the patient was admitted at hospital for convulsions). Notable is that all the recorded birthweights were high except one (G P 3030 g, born 1 month before term)

The siblings B. W P and J B W were according to the case records diagnosed as cerebral haemorrhages but the examinations and clinical course does not give any criteria for prenatal lesions or birth trauma.

Routine blood examinations (haemoglobin level, red and white cell cou t) have been normal in all cases. One patient, J B. W., had a high thrombocyte count. In the last two patients (cases 1 and 2)

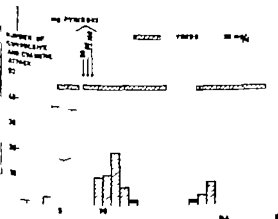


Fig. 2 Pyridoxine treatment during convulsive period in case 1.

thrombocyte counts therefore were made and showed normal values. This high thrombocyte count is probably irrelevant. Urinary examinations including protein and glucose content and urinary sediment have been normal. In the more thoroughly examined later cases there are normal values for serum sodium, potassium, chlorides, calcium, phosphorus, magnesium and copper

Hypoglycemia is a well-known cause of convulsions (6) but in our cases blood sugar was normal in all cases with the possible exception of M P (case 1) Further examinations revealed no abnormality in glucose metabolism and convulsions occurred with similar frequency when blood sugar rose to quite normal values.

Two types of disturbances in metabolism related to vitamin B_6 are known to cause convulsions in infants, deficiency and dependency (1 2, 4 8 11). The B_6-deficiency syndrome is due to insufficient intake and the tryptophane load test is positive. The B_6-dependency syndrome is familially occurring, the biochemical defect is unknown and the tryptophane load test is reported to be normal. Both these disorders are cured by pyridoxine treatment, the dependency syndrome requiring higher doses. A tryptophane load test was made in cases 1–6 with normal results. Only in cases 1 and – were this test done early during the convulsive period. The others were tested at 2, 3 7 and 9 years respectively The value of this test in B_6-dependency is doubtful (3). Our two youngest patients got pyridoxin treatment during convulsions up to 200 mg/day intramuscularly without any effect (Fig –) These doses are higher than those said to be effective in the B_6-dependency syndrome (10–80 mg daily according to Bejsovic *et al* 1967 (1) Thus, B_6-deficiency can be excluded and B_6-dependency is a very unlikely explanation to this convulsive disorder There were no signs of any of the other known disturbances in the amino acid metabolism

EEG-tracings were made in 7 cases and were normal except the 2 tracings recorded during or short after a convulsion. In these cases EEG was normal at a control 1 week and 10 days respectively after the convulsive period was over The recorded EEG-abnormalities were unspecific. Therapeutic trials with barbiturates and phenantoin were made without success in several cases.

There is no difference in sex distribution. There are seven male and seven female members of the family with the disease. It appears to be an autosomal dominant trait as shown by the pedigree In the last (5th) generation 6 out of 7 siblings were affected, but not their only cousin (son of J B W) In the first and second generations it is not known whether there were siblings with or without convulsions, but in the third and fourth generation all the children had the disease.

Rett & Teubel (7) described a family in 196– where 8 members in 3 generations had frequent convulsions which began on the third day of life and spontaneously disappeared in some week followed by quite normal psychomotor development. It is of interest, that also in this family some members had occasional convulsions at school age, mostly combined with febrile illness. There were no sex difference in these cases either and they seem more or less identical with the cases described in this report. A third family has been described from Norway (10) where 8 members had neonatal convulsions. B_6-dependency could be excluded and these patients also had normal psychomotor development.

The etiology of this disorder is at present entirely obscure. It seems to be an autosomal dominant hereditary disorder with convulsions during the neonatal period and a certain disposition for convulsions during childhood. It does not affect mental or motor development. An explanation could be that some enzyme system essential for cerebral metabolism is not fully developed at birth, but this is entirely a matter of speculation.

SUMMARY

A family is reported where during the neonatal period convulsions have appeared in not less than 14 members in five generations. It appears to be an autosomal dominant trait. The disorder is benign and the convulsions have disappeared in the first weeks with a few exceptions, where sporadic convulsions have appeared up to 10 years of age. The etiology is at present unknown and there are no signs of disturbance in electrolyte or sugar balance or in amino-acid metabolism.

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PROCEEDINGS OF PEDIATRIC SOCIETIES

FINNISH PEDIATRIC SOCIETY

Meeting Oct. 18, 1967

John Lind (Stockholm) *Placental transfusion and cardio-respiratory adaptation of the newborn infant*

The lecture was presented at the meeting of the Finnish Pediatric Society held in honor of the 80th birthday of Arvo Ylppö and has been published in *Ann Paed Fenn 14* 1 1968

Meeting March 29 1968
(together with the Finnish Otolaryngologic Society)

J Lumio *Investigation of hearing in young children and infants*

In Finland the first investigation of hearing is at present performed at the age of 7 years when the children start at school. However this investigation is not performed uniformly in the whole country Thus, some children have not the possibility of audiometric investigation, and some children are not at all investigated. According to present conception investigation of hearing should be performed much earlier preferably so that treatment could start already at one year of age.

There are some questions which should be discussed. Is it sufficient to investigate only infants belonging to a risk group or should all children be investigated? At which age should the investigation be performed?

At the age of 4 to 6 months an infant turns its head against a sound and there is still observation time left until the child is one year old. The health nurses could perform the investigation with the aid of standardized toys, but need special training.

According to an investigation performed by Sonninen in the Jyväskylä area about 0 6 per cent of the investigated children need closer investigation by a specialist. Thus in Finland about 500 children would need special investigations every year Many of these children have cerebral palsy or are in other ways disabled. The closer investigation should therefore be performed at the central hospitals, where cooperation with the pediatrician is possible. The treatment has to be organized and there are still many difficulties to overcome, especially the shortage of phoniatrists.

At the discussion H. Hultin told that in the parish of Espoo all 5 year old children have been investigated by an otologist. About 1000–1500 children have been investigated every year Audiometry was performed in all cases, and a closer investigation was performed in 10–15 per cent of the cases. An enlarged adenoid was the cause of impaired hearing in 46 per cent of the cases. For instance, in 1967 only 3 children with grossly impaired hearing were observed.

O Meurman expressed the opinion that the field investigation should be performed at the childrens wellfare centers, but he stressed the importance of training of the personnel. Otherwise to many cases would be sent to the central hospital for closer investigation.

E. I Wallgren